

THE HARVEY LECTURES

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BY

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PREFACE

THE Harvey Society was organized during the spring of 1905 through the efforts of Professor Graham Lusk. Its avowed object is the diffusion of the medical sciences by means of public lectures. It is the result of a feeling that the medical profession would welcome an annual series of lectures, dealing with what is generally considered the purely experimental side of medicine, and given by those who devote their time to experimental work.

The results of research work, both in this country and abroad, are generally published in specialized journals, of which a very large number exist. On this account, much that is of value to the medical practitioner, already overburdened with clinical literature, is either lost to him completely, or greatly delayed in reaching him. The Harvey Course is designed to remedy this condition. The lectures are not intended to be merely accounts of experimental work done by the lecturers, except in rare instances. They are rather to be a broad presentation, from the laboratory point of view, of subjects of general interest. The presentation includes a résumé of the experimental work done on the subject, and a critical review of this work in the light of the most recent advances. The lecturers are selected on account of a special adaptation through their own research work on the subjects presented by them.

It is the plan of the society to give an annual course of about ten lectures, during the winter months. Through the kindness of the Council of the New York Academy of Medicine, permission has been granted to announce the lecture course as being given under the patronage of the Academy, and the lectures are delivered in the Academy building.

PREFACE

In the course of the past year, the first of the Society's existence, thirteen lectures were given. The cordial reception they received has removed all doubt concerning the success of the undertaking and the desire for such a course.

The present volume contains the lectures of the first course.

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INTRODUCTION

THE first public lecture under the auspices of the Harvey Society was held at the New York Academy of Medicine on October 7th, 1905. The President of the Academy, Dr. Dana, addressed the audience, and was followed by President Graham Lusk, who spoke for the Harvey Society as follows:

“Gentlemen of the Medical Profession of New York: On the behalf of the Harvey Society, I beg to thank President Dana for the kind words he has spoken concerning our undertaking, and through him to thank the Academy of Medicine for its patronage, so helpful to the success of our newly-formed society.

“It is perhaps fitting to describe the reasons which have led to the formation of the Harvey Society.

“There exist to-day in New York two classes of medical societies. The first class is devoted to clinical work, and has existed since the foundation of the town. The other class is employed in research along the lines of experimental medicine. The societies of the latter class are of recent origin and may need a word of introduction. Seven years ago the Society of Physiological Chemists was established and still affords opportunity for the discussion of problems in an ever widening field. The Harvey Society is indebted to this sister association for a goodly subscription—the total amount of its surplus funds. The Society for Experimental Biology and Medicine was founded three years ago through the influence of Dr. S. J. Meltzer. Members of this Society must be workers along experimental lines. It is evident that the societies having clinical discussions for their aim must of necessity be of different character from those whose objects are exclusively experimental or theoretical. But it is also true that knowledge of the latter sort, if presented in a broad and liberal spirit, may be made of value to the members of all classes of the medical fraternity.

“Having in mind this idea of creating a common meeting ground, some of the laboratory workers in New York came together last spring and decided upon a new experiment. This experiment is the Harvey Society, founded for the diffusion of knowledge of the medical sciences through the medium of public lectures by men who are workers in the subjects presented. Dr. Theobald Smith has expressed our thought as regards the character of these lectures. He writes, ‘If I understand the scope of the society rightly, such a lecture should be prepared with great care, from the broadest and most advanced outlook, while being at the same time purely instructive.’

“In Berlin and in Paris men of science give public lectures with the wish of serving those busy with the art and practice of their profession. The Harvey Society is of this spirit. We would welcome the support of the notable audience of the medical profession of New York.”

THE THEORY OF NARCOSIS *

HANS MEYER, M.D.,

Professor of Pharmacology, University of Vienna.

I ESTEEM it a high honor as well as a great pleasure to address you on this occasion and to have been asked to open the first course of lectures of the Harvey Society. The establishment of these lectures is additional evidence, if such be required, of the great interest displayed by American physicians in theoretical conceptions and in scientific research, and, in addition, of an earnest effort to encourage and diffuse such knowledge.

I look, however, on the invitation extended to me as evidence of your friendly and fraternal sentiments toward the whole body of German scientists, and I may be permitted on their behalf to offer you an expression of their cordial appreciation.

Following the suggestion of your president, I have selected as a theme for this evening's lecture a subject which, for a long time, has repeatedly attracted and interested not only pharmacologists, but biologists as well, namely, the relationship between the pharmacologic action of a drug and its recognized chemical or physical properties. The solution of this problem presents great difficulties. Even with a knowledge of the chemical and physical properties of an active substance it is yet impossible, without further knowledge, to determine which of these properties is responsible for the specific action on the animal organism. And this the more so, since we do not know the chemical point of attack in the organism, and hence can not know the nature of the chemical reactions which occur between poison and protoplasm. Only in one way can we reach a conclusion that admits of probability. If we find a large series of different

* Lecture delivered October 7th, 1905.

substances with different chemical and physical properties, which all possess identical or else very similar pharmacologic actions, then we can fix on the chemical and physical properties common to all and on which the common pharmacologic action naturally depends. So, for example, out of a mass of keys different yet all able to open the same lock we can determine which part of each key is the essential one, what form common to all fits the lock. And it is hardly necessary to point out that from this we can obtain an insight into the construction of the lock itself. In our case this means an insight into the chemical organization of protoplasm.

The first experiment in this direction was made by two English investigators—Crum-Brown and Fraser. They discovered the notable fact that practically all the ammonium bases, that is, organic bases in which the pentavalent nitrogen is connected with four valencies to carbon, exercise the same pharmacologic action, regardless of other differences in their constitution and nature; the action in this case being the same as that of curare, a paralysis of motor nerves. This definite relationship has been confirmed by many investigators, but the explanation for it is still lacking. It appears to me possible that the strongly basic character of all these ammonium bases is the chief factor. They are much more strongly basic than alkaloids and even sodium and potassium, and the few exceptions, such as betain, antipyrin and others which do not possess this strongly basic character, are also without the curare-like action.

Another series of experiments along this same direction, in connection with the action of the neutral alkali salts, has been conducted by Hofmeister. He has shown that all the effects of these salts, including their laxative and diuretic actions, may be explained by their physical properties, their diffusibility and osmotic strength.

This brings us to a third especially large group of substances whose actions are all identical in principle. I refer to those substances which are commonly designated anesthetics. To this group belong bodies quite distinct from each other chemically;

alcohols, aldehydes, ketones, esters, ethers and numberless others. They all possess the common action of depressing the central nervous system. Wherein is the relationship? On which of their common properties is their narcotizing action dependent? The chemical composition of the nervous system itself gives the first clue for an understanding. It differs from all other tissues in its richness in fat-like constituents, and on the basis of this peculiarity Bibra and Harles attempted many years ago to explain the action of anesthetics. They found that the anesthetics dissolved ordinary fat, and, as a result of a quantitative estimation of the fat content of the brain of a normal and narcotized animal, assumed that the anesthetics directly removed fat-like substances from the brain. But these conclusions could not be confirmed. From another standpoint, however, Hermann arrived at a similar opinion of the action of narcotics. Hermann had discovered the presence of lecithin in the red blood cells, and since the anesthetics, such as ether, chloroform, etc., dissolve the blood cells, he explained this by their power of dissolving lecithin, and pointed out the parallelism between this process and the narcosis of the central nervous system. In both of these hypotheses there appeared to me to be an element of truth, and in order to establish this and define its character I myself instituted a series of experiments.

I started with the following assumption: If fat-solubility is indeed a necessary condition for narcotic action it is to be expected that all indifferent, fat-soluble substances must act as narcotics if they can enter the cells, and that, on the other hand, if by any circumstance they lose their fat-soluble property, then they must also become inactive. I have tested this assumption by investigating a series of substances made by combining components which in themselves had no narcotic action, but whose combinations were soluble in fat. As examples may be mentioned the amides of organic acids. These amides are neutral compounds which are soluble in fat and which all possess the typical narcotic action, with, however, one single exception, carbamide, and this particular one is insoluble in

fat. Another group (comparable to the amides) is composed of condensation products of glycerin; the chlorhydrins, acetins and glycerin-ether. These also are soluble in fat and act as narcotics. All these substances, however, are very readily split up by hydrolysis into components insoluble in fat and on such decomposition lose immediately their narcotic power.¹

Now, if from these and similar observations the conclusion can be drawn that the solubility of an anesthetic in fat is certainly one of the conditions for narcosis, the further question presents itself whether this condition is an essential one and whether it can be utilized as a measure of narcotic power. Were this the case, a quantitative relationship between narcotic power and solubility in fat must exist. But it is obvious that other factors must influence the action of narcotics in the animal body, for their affinity for the watery components of the body, as well as that for the fat-like constituents, must be considered. According to their relative solubility in the fat-like and non-fat-like constituents of the body they will distribute themselves between those constituents. So, for example, such substances as are very little soluble in water will dissolve for the most part in the fat-like constituents.

Richet had previously made the observation that the anesthetics which are little soluble in water possess a marked narcotic action, and he regarded this relationship as a general law. As a matter of fact, however, from this single relationship no general law can be deduced, for substances such as alcohol and chloral, which are both equally soluble in water, possess very different narcotic powers. The proper expression of the law must, therefore, be that the distribution relationship, the so-called distribution coefficient, of the narcotics between fatty and watery solutions, is the determining factor of narcotic action. To test the correctness of this hypothetical law I have determined the strength of action of a large number of different narcotic poisons by estimating the smallest molecular concen-

¹ The amides yield fatty acids and ammonia salts, the glycerin derivatives, glycerin and acetic or hydrochloric acids.

tration of their solutions which was sufficient to induce narcosis of small fish and tadpoles placed in them. A second series of experiments was then carried out with the same substances for the purpose of determining their distribution coefficient between water and fat. A mixture of water and oil was used to estimate the relative quantities passing into these two substances. The comparison between the distribution coefficient so obtained and the narcotic strength of the narcotics did, as a matter of fact, yield the expected result, as a glance at the following table reveals:

TABLE SHOWING THE RELATIONSHIP BETWEEN THE DISTRIBUTION COEFFICIENTS F/W AND THE CONCENTRATIONS, EXPRESSED IN GRAM MOLECULES, OF SOLUTIONS EXERTING EQUAL NARCOTIC ACTION.

	F/W	Concentration
Trional	4.4	0.0013
Tetranal	4.0	0.0018
Butylchloral	1.6	0.002
Sulphonal	1.1	0.006 ?
Bromalhydrate	0.7	0.002
Benzamid	0.6	0.002
Triacetin	0.3	0.010
Diacetin	0.23	0.015
Chloralhydrate	0.22	0.025
Æthylurethane	0.14	0.025
Monoacetin	0.06 ?	0.02 ?
Methylurethane	0.04	0.4
Æthyl alcohol	0.03	0.5

With an increase in the distribution coefficient there occurs an almost parallel increase in the narcotic strength, that is, decrease in the molecular concentration necessary for narcosis. The few departures from the general rule which occur in the table can be explained by the naturally inexact method of estimating the narcotic power.

A further proof of the correctness of the view described may be offered. It is known that the solubility of most substances in water and fat changes in a different way with variations in temperature. The distribution coefficient is also variable according to temperature. It must be expected, then, that the narcotic strength as well will vary with changes in

temperature. And this is the fact. I examined six substances, of which with higher temperature three gave higher and three lower distribution coefficients. And it was found that in exact accordance with the rise or fall of the distribution coefficient so the narcotic strength rose or fell, so that tadpoles which were just narcotized by a certain chloralhydrate solution at 30 degrees C. were aroused and quite active on cooling to 3 degrees, and on subsequently warming to from 25 to 30 degrees again passed into narcosis. From this the direct dependence of narcosis on the physical relationship of the narcotic to the fat-like substances, the lipoids of the body, and the watery constituents seems to be definitely proved.

As a result of all these studies we arrive at the following explanation of narcosis: The narcotizing substance enters into a loose physico-chemical combination with the vitally important lipoids of the cell, perhaps with the lecithin, and in so doing changes their normal relationship to the other cell constituents, through which an inhibition of the entire cell chemism results. It also becomes evident that the narcosis immediately disappears as soon as the loose, reversible combination, which is dependent on the solution tension, breaks up. It follows further that substances chemically absolutely indifferent, as the volatile saturated hydrocarbons, can act as narcotics.

Quite in opposition to this idea, it has been frequently put forward and accepted that the breaking up of the narcotics, with a chemical action of definite atomic groups thus set free, as the ethyl group, for instance, is responsible for the narcosis. But even in the case of sulphonal and its related sulphones, from which this idea originates, it can be shown that the action is induced by the entire unchanged molecule, and that the lack of activity of certain sulphones is due not, as is generally believed, to their not being broken up, but to a low distribution coefficient.

This simple theory also explains the fact that all structures capable of stimulation, not only the cells of the nervous system, but all others, and all plant cells as well, are depressed by the narcotic members of this series, for in all living cells lecithin,

a lipid body, is to be found. And, indeed, the establishment of the fact that the effect on the lipoids by narcotics, such as ether and chloroform, is such as to immediately inhibit the vital processes of the cell, shows us that these lipoids are among the constituents essential to the life of the cell. Moreover, by establishing this fact it seems to me that the general biologic significance of the theory becomes apparent.

That many narcotics induce not pure narcosis alone, but often show other distinct actions, as, for example, the occurrence of convulsions, which quite overshadow any narcosis present, is easily to be understood when one remembers that the narcotics may possess an affinity not only for the cell lipoids but for other cell constituents as well, and through some union with these, concomitant effects quite different from narcosis may be induced. This occurs, for instance, in the case of the phenols, whose narcotic action is thrown into the background by the appearance of clonic spinal convulsions.

No attempt is made to explain every type of narcosis by means of the theory presented here. It is very probable that some other disturbances in chemical equilibrium can occur in the cell and inhibit the performance of its function and that substances such as morphin are narcotic through their relationship to other points of attack than the "alcohol lipoids"; and most probably the same can be said concerning the very remarkable narcosis from magnesium salts, lately discovered by Meltzer.

I desire to add in conclusion that shortly after I had published my theory of alcohol narcosis the physiologist Overton published experiments which, carried out independently of mine and from a different point of view, in fact with somewhat different methods, brought him to an identical conclusion, *i.e.*, to a similar theory of narcosis, so that he has confirmed my work and accepted the formulation of my theory literally. I take this as a strong and gratifying argument for the correctness of our assumption.

MODERN PROBLEMS OF METABOLISM*

CARL VON NOORDEN, M.D.,

Professor of Medicine, University of Vienna.

YOUR president has conferred on me a great honor in asking me to deliver one of the opening lectures of the new Harvey Medical Society of this city.

It is your aim that from this well-established center waves of scientific stimulation for research work may arise and reach not only the circles of the professional workers of this city, but even those of the whole country. At this moment, when a society, promoted under such favorable auspices, opens its career by a course of lectures, I think it is opportune, not only to recount the results of investigations already completed, but principally to consider those problems which still await solution.

I am perfectly aware that in doing so I must renounce giving to my hearers the harmonious impression which a well-worked scheme calls forth; for I am to touch manifold subjects and points of view which stand wide apart and in no organic relation to each other.

Even in confining myself to a very small sphere of the problems of metabolism, a complete and exhaustive representation of such will be impossible. Only a small selection can be made, and even this will savor of arbitrariness.

I may touch, perhaps, on several subjects which to you appear quite unimportant, and, on the other hand, I may omit many points which are of recognized importance. I expressly remark, therefore, that I shall mostly confine myself to questions which enter into my own program for future investigations on the problems of metabolism. If, as a result of my communications,

* Lecture delivered October 14, 1905.

you gain the impression that details of the problems are thrown together by arbitrariness or by chance of selection, I hope that, on the other hand, the personal factor will be the joining link for compensating such disadvantages.

A short retrospect of the history of several problems of metabolism may form a useful preface. All the first investigations, decades ago, were directed toward the recognition of the quality of the chemical changes in the body. The substances which resulted from the breaking up of the tissues of the body and of the ingested food were the earliest to be demonstrated. The end-products of animal metabolism were determined. Most important rules were discovered concerning the production of CO_2 , urea, uric acid, kreatinin, indican and hippuric acid, etc. Among the normal end-products, many substances were found which appeared only under certain conditions, and were regarded as characteristic for particular diseases. As examples of such substances I may mention sugar, the various types of albumins, peptone, leucin, tyrosin, lactic acid, cystin, etc. Following this period, in which the names of Wöhler and von Liebig stand out prominently, came the second era, viz., that of pointing out the quantitative changes of metabolism. First introduced by Bischoff, the work in this branch of investigation was carried on and thoroughly established by Carl von Voit and von Pettenkofer and their pupils. Originally confined to the physiologic circumstances in animals and men, this "quantitative study of metabolism" has since obtained new triumphs in its application to clinical medicine and to the study of pathologic processes. It is scarcely twenty years since these investigations commenced, and already, both in the physiologic and in the clinical laboratory, these quantitative estimations are being placed in the background, while attention is being directed to the newer field of the intermediary processes of metabolism. The finest and best work of late years relates to these questions. Hence to-day the investigations on metabolism approach again in character to those of the first period; but what then appeared impossible is now being attacked from all sides. Then one had to be satisfied with a knowledge of the

end-products only; to-day one endeavors, through the prominent discoveries in chemistry, to make clear the intermediate stages, through which the metabolites pass to their final conditions. An infinite number of new questions is thus presented by the recent advance in physiologic and pathologic chemistry.

A number of important questions, which are of interest to the physiologist and pathologist alike, however, were left unsolved during the earlier periods of quantitative estimations, and it is only now that—thanks to the better technic of recent times—exact measuring methods are available for their investigation.

First of all, there is the question of the metabolism of energy. Since the time of Voit and Rubner it has been customary to express and to measure body “energy” in terms of calories. In part through the relation of the body weight to the necessary intake of food, and in part from the amount of oxygen consumed and of CO_2 expired, certain average figures have been determined. When an adult man is in a condition of complete muscular rest, from 22 to 24 calories per kilo of body weight are necessary during each twenty-four hours; with usual light work, from 32 to 36 calories are required. The daily food must have these calorific values if the weight of the body shall neither increase nor diminish. With the increase of muscular work, the amount of energy consumed increases in certain proportions, and these latter have been sufficiently ascertained. We know also that children require a relatively high, and old people a relatively low, exchange of energy.

Still, all these are only average numbers and they require the further support of numerous careful and exact observations. Even the most trustworthy figures, obtained by the use of methods of whose accuracy there is not the slightest shadow of doubt, showed that under exactly the same conditions a difference of from 20 to 25 per cent. arose between single individuals; this can only depend on the so-called individual factors. In future, however, this difference may not be slurred over by the use of the mystic word “individuality”; we must endeavor to make clear the reasons for the rise above the average

in oxidative processes in one person, and the fall below the average in another. Such information would provide us with a clear—I might even say a mathematical—insight into the condition which we now designate by the term “individuality.”

An important by-question which arises in regard to the physiology of nutrition, is the problem of the influence exerted on the consumption of energy by the respective constituents of the food.

Certain experiments which Max Rubner and Ed. Pflueger have carried out on animals, tend to show that when the food contains an excessive quantity of proteids the energy-exchange rises considerably above the average. The energy production appeared to rise higher than was necessary for the muscular work done and for the maintenance of the body warmth. These results remind one of the old theory known by the name of “Luxus-consumption,” if it does not even entirely compass it. They are too few and insufficient to revive the old hypothesis, which we have long known to be erroneous. As, however, one of the bases of the new science of nutrition is touched by it, the point should be thoroughly cleared up by new and better experiments on human subjects. If the excess of proteid intake really exerts a marked influence on the oxidative processes of the human organism, then we must change many of our views and explain differently a number of former experiments in metabolism. Up to now we trust that not the kind and the amount of food but only the internal and external bodily work rules the extent of the oxidation. The question is not a theoretical one only. Recently manifold endeavors have been made to shake the old standard numbers for the albumin intake of healthy men settled by the school of Voit. It would be water on their mill could the supporters of vegetarianism from whom these endeavors originate, prove their contention, that large amounts of albumin raise the consumption of energy to an unseemly, that is to say, to an unnecessary and prodigal, extent. The theory of vegetarianism would also receive a specially strong support were it possible to confirm the oft-spoken assertion that the prodigal expenditure of energy only follows an

excessive intake of animal albumins and does not result from a similar quantity of vegetable albumins. A few experiments we made lately turned against the theories of Rubner and Pflueger.

Of greatest interest and importance are, of course, those alterations of the exchanges of energy which occur in various diseased conditions. Single and occasional former investigations excluded, we first commenced only about ten or fifteen years ago to busy ourselves with these matters. One single important fact is thoroughly established, viz.: the increase of the energy exchange which follows the administration of thyroid gland substance. This observation, which was made in my clinic by my former assistant, Prof. A. Magnus-Levy, was suggested by the practical experiences of Yorke-Davies and Leichtenstern on the influence of thyroid gland tablets on obesity. Later, Magnus-Levy discovered a similar increase in the transformation of energy in exophthalmic goiter and a decrease in myxedema. But these are the only diseases in which, up to now, spontaneous changes in the output of energy are known to occur. Thus the studies—I might call them preliminary—which have hitherto been made on the extent of the processes of oxidation and the amount of nutriment necessary in diseased conditions, afford sufficient reason for the use of our improved methods in further investigations in this field. Many of these problems are of great practical importance for bedside treatment. Next, there is the old question of how great the metabolism energy is in people who are run down by chronic disease or by insufficient nourishment. Do these persons require the same amount of food as do healthy individuals, reckoned per kilo of body weight, or do their bodies diminish the extent of exchange on some self-regulated plan? It is certain that the albumin metabolism is diminished. It has even been asserted that the total production of energy also is diminished, but on this we are as yet without definite proof. My preliminary observations point to the contrary, but the question has not been investigated with scientific exactitude. The extremely painstaking and brilliant work of Neumann in Kiel,

and of Chittenden in America, which has demonstrated the surprising extent to which the food of an adult man may be diminished without affecting the capacity for work and without altering the nitrogenous equilibrium of the body, leaves untouched this particular question.

Obesity is quite the contrary. For a very long time it has been asserted that there are two forms of obesity. One type is said to result from an excessive intake of food or from insufficient muscular exercise; the other is said to arise from an endogenous retardation of metabolic exchanges. The question is of great theoretical interest, but, as every one must admit, it is also of marked practical therapeutic importance. Since I first approached the matter, some twelve years ago, by investigations on the respiratory exchanges, the question has been constantly discussed. Some differences exist between the results of clinical observation and of laboratory experiments. Clinical reports indicate the occurrence of cases in which the obesity is due only to abnormal lowering of the oxidation, that is, to a diseased state of the protoplasm. Scientifically exact experiments, however, have failed to discover such relations. The results of some work done in the clinic at Basel seemed to point to abnormal low oxidative changes during muscular work and during the digestive processes of obese persons, but they must be discounted by the fact that the methods of estimation employed were not free from objections; correct deductions from them are therefore impossible. I am convinced, however, that with the advent of more satisfactory methods the views of the practitioners will be confirmed by laboratory experiments.

Since the earliest days of investigations on metabolism, the question as to the energy exchanges in fever has received attention. That the albumin exchanges are increased is quite certain; toxic influences are the reason. But why does the patient waste during the periods of fever? Why does he also lose so much of his body fat? As a matter of fact, in every case of long-continued fever, we observe an enormous loss of weight, even if we endeavor to avoid this loss by the administration of rich and nutritious foods. Does the cause lie in the fact that

in spite of all our care an individual can not ingest the normal average calories of the food, since the digestive organs during fever are unable to take in or to digest the necessary amount? Or do the oxidative processes in the fever periods rise markedly above the normal? If this is the case, the food requirements of the fever patient will not be satisfied by ordinary quantities; the amount of food sufficient for a healthy individual would not prevent the patient wasting during the stages of fever. The practitioner of earlier times did not doubt that fever was always accompanied by a substantial increase in all the processes of oxidation. The exact investigations on metabolic changes which have been made during the last decades do not, however, confirm these ideas. These consist, in particular, of the works of Senator and some investigations by F. Kraus and by the pupils of Zuntz. If we thoroughly and critically read through these works, we find that they are full of contradictions and by no means permit of any final conclusions being made. The technic of to-day promises, however, a satisfactory and objection-free solution of this old problem. Still, the working out of the matter is naturally dependent on clinical material, and, unfortunately, the majority of hospitals to-day are not equipped with the necessary apparatus.

Among other diseases in which the energy exchanges should be further investigated, I may mention diabetes mellitus. In slight cases, the relations are simple and undisputed. Such cases do not exert any influence on the energy exchanges. For a long time, however, it has been supposed—and lately the assertion has been revived on many sides—that in severe cases of diabetes the production of energy, and consequently the food requirements, are distinctly diminished. It has been calculated that in these patients the daily energy needs are satisfied with from 18 to 20 calories per kilo of body weight, while the healthy person requires from 34 to 36 calories under parallel conditions. The question is of great practical importance, because a clear conception would be of real assistance to us in the difficult dietetic treatment of diabetes mellitus. I do not admit that the just-mentioned figures, concerning the diminished production

of energy in severe cases of diabetes, are quite correct; and I am of the opinion that the few previous exact observations on the production of CO_2 and the consumption of oxygen, are quite sufficient to prove this. Anyone who possesses a large respiratory apparatus can definitely settle the entire question in a few days.

We leave now those questions which are intimately connected with the transformation of energy, and turn to another very interesting and important problem, relating to the metabolism of albumin. Earlier experiments on animals and recent investigations on human subjects have taught us that an excessive amount of food compels a retention of nitrogenous substances in the body. The usual nitrogenous equilibrium is disturbed; a smaller quantity of nitrogen appears in the excreta than was present in the food. This retention of nitrogen may be attained by the administration of large amounts of albumin, but much more thoroughly and surely by a simultaneous excess of fat or especially of carbohydrates. The albumin-sparing properties of the two latter substances, of course, are well known. The ultimate effect of such over-nutrition is always an increase in the total quantity of fat. We apply this knowledge therapeutically in our "feeding cure," etc. But regarding the nitrogen there was until a short time ago the opinion that in spite of such an excessive nutrition, the nitrogen retention was only slight in quantity and short in duration—at least so far as well-nourished adults are concerned. It was taught that the body always endeavors to maintain a nitrogenous equilibrium so that, in the case of over-nutrition while the excess storing of fat may continue for a long time, a similar storing of proteids is soon stopped. In certain cases, however, the storing of body proteids seemed to be both extensive and long continued, as for instance, during the period of body growth, or after chronic exhaustive diseases, or after periods of lowered nutrition—it is always during the new growth of tissues. The occurrence of considerable nitrogen retention has recently been noted, apart from the conditions just mentioned. In a case of my own, I found that in two months not less than 370 grams of nitrogen

were retained. Expressed in terms of meat this is more than 11 kg. of flesh. Is this retained nitrogen really built up into pure albumins and protoplasmic substance? Our general knowledge tends to indicate otherwise. We know that excessive feeding produces obese, but never athletic, individuals. *A priori*, it is very improbable that the nitrogen retained during excessive nutrition indicates the formation of pure albumins or a new formation of tissue substance. Perhaps the nitrogen only exists in the form of nitrogen-containing fragments of the large molecules of albumin, which are held for a time and are then cast off at a later period. In favor of this supposition there is the fact, that when the period of excessive nutrition is stopped, it is usual for an enormous quantity of nitrogen to appear in the urine.

The exact form in which the nitrogen is retained within the body is still, however, entirely unknown. This question is important, because a knowledge of it would throw light on the changes which the molecules of albumin undergo in the body.

This problem leads to a consideration of the intermediate stages of metabolism, the special field of modern physiologic chemistry.

Naturally, most questions of the "intermediary" metabolism concern themselves directly or indirectly with the fate of the albumin molecules; with their disintegration as well as with their synthesis. It seems that the synthesis of albumin in the body may originate from much simpler molecules than we could conceive of until lately. By intense and long-continued tryptic digestion of albumin, the latter has been broken up until the solution no longer yields the biuret reaction. In spite of this, the administration of the products of such digestion to animals serves for the substitution of pure albumins and for the maintenance of nitrogenous equilibrium.

In close theoretical relation to this brilliant and important experiment of Otto Loewi, stand those considerations which are bound up with the discovery of erepsin in the walls of the alimentary canal. This ferment splits up the albumoses and peptones into simpler substances and, in particular, splits

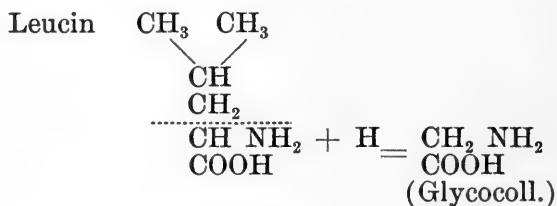
off the amino-acids. Hence, it has been assumed that this action represents the regular arrangement of processes, that the organism normally lives on the amino-acid mixtures, and that from these basal substances are formed the albumins which ultimately circulate in the blood stream. Such a sweeping conclusion, however, is a little too previous, for it has been shown recently that erepsin occurs in all the organs of the body and thus is not specific for the alimentary tract. The ferment, which *in vitro* is able to split up the albumoses, may synthetically form albumoses from amino-acids when acting in the intestine. Such reversibility of ferments is already known. These considerations appear to indicate, therefore, that the real function of the intestinal mucosa is not to break up the albumoses into amino-acids, but, on the contrary, to build up albumoses and similar substances out of the amino-acids, which pass into the intestinal wall from the lumen. The question is certainly an available one for further experimental investigations. First of all, the living and surviving intestinal mucosa must be allowed to act on a mixture of amino-acids. This experiment has not yet been made. It is one, of course, which is very important for our ideas concerning the assimilation of albuminous substances. If the investigation yielded positive results, there would be a remarkable analogy between this process and that of the synthesis of fat by the intestinal cells. Concerning the fate of fats, we know (1) that a fat-splitting ferment (lipase) is present in the intestinal wall; (2) that by the aid of this ferment there also occurs in the intestinal wall a synthesis of fat from fatty acids and glycerin; (3) that *in vitro* this synthetic process can be reproduced by the aid of lipase. For these brilliant and important investigations we are indebted to your own countryman, Dr. Loevenhart.

These questions are of great significance in practical dietetics, since they bring into greater application the until now almost entirely neglected amino-acids. In particular, rectal feeding would receive a new impetus. Among the amino-acids there are many substances which are less irritable to the mucosa of the large intestine and are more easily absorbed than the

usually prescribed albumoses and peptones. We have already commenced investigations on this point.

Associated with the amino-acids, which represent the chief nitrogen-containing group of the albumin molecules, are many other questions, only a few of which can be touched on here. The chief of my clinical laboratory, Dr. G. Embden, has lately made an investigation on the amino-acids under physiologic and pathologic conditions. What I have to say on this point is due chiefly to the important theoretical and analytical work of Dr. Embden.

First, I have to mention that glycocoll probably can be split off from all the amino-acids, the chains of the higher constituted amino-acids being broken up between α and β C-atom.

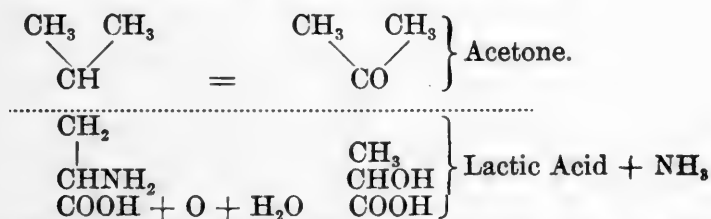


This process, it would appear, plays a great part in the organism. Recent investigations in my laboratory have shown that glycocoll is normally present in all urines, and in such quantities as to approach up to 1 per cent. of the total nitrogen output. This well-grounded observation is striking, because we have for a long time known that glycocoll introduced into the stomach is very easily assimilated and reappears as urea in the urine. The formation of glycocoll within the body must be very large, if the kidney is able to catch and to eliminate some portion of it. This production of glycocoll from the higher amino-acids may also explain how it is that the body always has glycocoll at its disposal for coupling or combination purposes. I recall to mind the instances of hippuric and glycolic acids. Further experiments must be performed, however, in order to determine whether or not the administration of large quantities of the higher amino-acids is followed by the appearance of a supernormal amount of glycocoll in the urine.

If the formation of glycocoll from the higher amino-acids

takes place in the manner which our preliminary investigations suggest as being probable, then a new light will be shed on the question of the formation of sugar from albumins. Since it has been established that higher amino-acids, such as alanin, form a definite source for sugar (G. Embden and H. Salomon), G. Embden, working in my laboratory, has shown that after the removal of the pancreas in dogs, sugar is formed from glycocoll equally as it is from alanin and other higher amino-acids. Glycocoll seems to be one of the most prolific sources of sugar that we know of. This fact, taken in connection with the previously mentioned conditions of glycocoll formation out of other amino-acids, explains at one stroke the question of sugar formation from albumins, and effectively removes those objections which have been heard during the most recent years.

Glycocoll, however, does not seem to be the only amino-acid from which sugar can be rapidly formed. Our attention has also been directed specially to leucin, for the reason that of all the amino-acids leucin occurs most largely in the majority of the proteids of food. We are quite certain that lactic acid can be produced from alanin, and that in fact this procedure takes place within the body. A similar possibility obtains for the formation of lactic acid out of leucin. Theoretically, on the addition of water and oxidation, leucin breaks up into acetone and lactic acid, while at the same time amides are split off. Only just recently attention has been drawn to how often this process occurs in the chemistry of animal tissues and how important it is. In this case the chain of C-atoms is broken between the β and γ atom. You see, there are different possibilities of disintegration of the same molecule.



In confirmation of these theoretical possibilities, we have just proved that the "surviving" liver always excretes some acetone

into perfused blood, and that the amount of acetone considerably increases when leucin is added to the inflowing blood. At the same time, as we have determined with certainty the formation of lactic acid from leucin, we are met with a new problem, associated with the as yet unknown changes during the passage of carbohydrates through the body. We certainly know the end-products of carbohydrate disintegration—carbonic acid and water—but in regard to the intermediary metabolism of carbohydrates and the manner in which those end-products are produced we are still in the realm of theories. Entirely disconnected facts are alone our guides. One of the earliest theories related to the formation of lactic acid from carbohydrates; but until now no satisfactory proof of this was given to us, at all events so far as muscular tissues are concerned. Many physiologists consider the lactic-acid formation in muscle to be due to post-mortem changes. The modern technics, which have advanced perfusion methods to a remarkable extent, will make possible a definite solution of the problem; until now, it is only solved so far as the liver is concerned. In my laboratory, Embden and Almaggia have thoroughly demonstrated that lactic acid ensues in fact from disintegration of carbohydrates by the liver. This result arises from the action of a ferment and, as all our experiences with organic ferments indicate that the action of these ferments is reversible, so this procedure may also take place in the reverse way. As a matter of fact, we know that very often the administration of lactic acid to individuals affected with severe diabetes, and more especially to dogs after removal of the pancreas, is followed by an increase in the glycosuria. We also consider lactic acid as a rich source of glycogen. These few available facts lead to the following hypothesis:

A part of the sugar which is broken up in the muscle circulates in the blood as lactic acid; the lactic acid passes to the liver and is there rebuilt up to carbohydrate and eventually reaches anew the muscles in the form of sugar. With this conception of the intermediary stages and circulation of the carbohydrates in the form of lactic acid, some well-known facts

are in full agreement. After extirpation of the liver, sugar disappears from the blood stream and lactic acid makes its appearance. Another remarkable fact may also be explained on this hypothesis. When the pancreas is removed from birds, glycosuria does not result. In these animals, lactic acid is not bound to be regenerated into sugar, but with the addition of ammonia, it can form uric acid. If this view be a correct one, then the uric acid of the bird is partly a derivative of sugar. I advance this theory, of course, only in the form of an hypothesis; it has, in any case, the advantage of promoting further investigations on the intermediary stages of carbohydrate metabolism and of providing a new aim and a definite proposition for further proof.

I have already mentioned that, theoretically, acetone may be produced from leucin, and that we have been able to demonstrate this procedure by experiments on animals. This result is very remarkable, since the opinions of to-day designate the fatty acids alone, and the lower fatty acids in particular, as the source of the acetone bodies, and because until now we have always accepted the oxybutyric and diacetic acids as the necessary precedents to acetone. This latter view thus requires correction, although our experiments in no way show that in the formation of acetone leucin plays an important figure in respect to quantity. At all events, it indicates that the acetone question can not yet enter into a condition of rest. Also the problems of the formation of acetone from fat and the hindrance to the production of acetone through the simultaneous oxidation of carbohydrate, are still sufficiently enigmatical and can not be solved until we know much more about the intermediary disintegration of fats and of carbohydrates than we do up to this day.

With this I wish to conclude my survey of modern problems of metabolism. As I stated at the commencement of the lecture, it has been necessary to roam over a large amount of ground and to consider subjects that are but slightly related to each other. You will observe that to-day we are busying ourselves in a much more intimate manner with the details of metabolic

processes than in not very remote periods was deemed either necessary or possible. Already the little that has been mentioned here is more than the working powers of one single man can master; but on all sides we see new young energy pouring into this interesting and important branch of medical investigation, in order to harvest this inexhaustible field. We greet it with joy and with satisfaction. The results will not be long in coming.

We are all convinced that these marked steps into the wonderland of animal metabolism will not only advance the theoretical science, but, as we have always seen, that every advance in physiologic and pathologic chemistry has been followed by improvement of our bedside treatment. The achievements of the dietetic treatment of diseases have gone hand in hand with the advances in theoretical investigations. If we compare the progress in dietetics that has been made during the last decade with the wonderful successes of the surgeon, the medical clinician no longer need feel either shame or envy. In the same period a vast amount of work has been done by the internist in regard to therapeutic matters. The close relations which have been maintained between the progress in clinical bedside treatment on the one hand and physiologic and pathologic chemistry on the other, have been very fruitful indeed, and still fruitful will remain.

Great problems still await solution and rich outside help is necessary thereto. With confident expectation, medical science looks to this country, in which in recent times numerous ardent and honest research-loving young workers have entered into the service of problems of metabolism, and in which the riches and the munificence of its inhabitants more than elsewhere have provided that external aid which has made more easy the prosecution of great and far-reaching investigations. I close with the prophetic words of our Goethe:

“Amerika, Du hast es besser
Als unser Continent der Alte.”

ON TRYPANOSOMES*

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UNTIL very recently the bacteria or plant organisms have been given a preponderating and almost exclusive rôle in the production of infectious diseases. The studies of the past few years, however, have brought to light another group of organisms which play an exceedingly important part in the causation of diseases peculiar to the warm countries. Unicellular forms of animal life are to-day the recognized causes of a large number of diseases whereas but a few years ago they claimed but scanty attention. The pathogenic protozoa, in a remarkably short time, have risen from an obscure to a commanding position by the side of the pathogenic bacteria.

Under the head of *protozoa* are classed: First, the *trypanosomes* which are met with free in the blood plasma; second, the *hemocytozoa* which find their habitat within the blood cells and are represented by the malarial organisms in man and by related forms in the lower animals; also by the piroplasmata found in Texas fever and allied affections. And, third, the *amœbæ* which are found in the intestine in dysentery. Many other forms of pathogenic protozoa are known but these are of relatively little interest compared with those mentioned above.

Although the first of these, the trypanosomes, have acquired special importance during the past decade, it is nevertheless an

* Lecture delivered November 4, 1905. The lecture was illustrated by a large number of lantern slides of which only a few can be reproduced in connection with this paper. The author wishes to acknowledge the courtesy of the Journal of the American Medical Association for the loan of the cuts of the illustrations which are given in the text.

interesting fact that the first representatives of this group were described more than sixty years ago. The credit of the discovery of this group belongs to Valentin who, in 1841, found the first trypanosomes in the blood of salmon. In the following year a similar organism was found in frog's blood and it was to this that Gruby gave the generic name *trypanosoma*, implying a screw- or auger-like body, the name being suggested by the peculiar motion of the parasite. It will be seen from this that the discovery of these organisms antedates that of anthrax (1849) and of the *Spirillum Obermeieri* (1873).

Up to 1850, trypanosomes had been found in the blood of several mammals, notably field mice, moles and rats, but these observations were eventually lost sight of, and it was not until 1877 that they were rediscovered by Surgeon-major Lewis at Calcutta. It was the interest aroused by the findings of Lewis that led to the discovery by Evans in 1880 of a trypanosome in the blood of animals afflicted with a disease known in India as surra. This organism, now designated as *Trypanosoma Evansi*, is consequently the first known really pathogenic trypanosome, inasmuch as the organisms met with prior to that time are commonly looked upon as harmless parasites.

During the following fifteen years, although many observations were made upon the trypanosomes in rats, fish, frogs and birds, they attracted but little attention, largely because of the all-absorbing interest in the study of bacterial diseases. The past decade, however, has witnessed a truly remarkable progress in our knowledge of the diseases due to protozoal organisms and the credit of bringing about a just recognition of the etiological rôle of trypanosomes belongs to Colonel David Bruce, of the British Army Medical Service. In 1894 he began the classical investigation of the terrible tsetse-fly disease or nagana of Zululand, the results of which study were published in 1895, 1897 and 1903. He showed that this disease was due to the presence in the blood of a trypanosome now known as *Tr. Brucei*, similar to that studied in surra by Evans and Lingard; that the disease was transmitted from the infected to healthy animals by the bite of the tsetse-fly, *Glossina*

morsitans; and that the persistence of the disease was due to the presence of the parasite in the large game which consequently acted as a reservoir for the virus. The trypanosome which he discovered was transported to England by Dr. Wag-horn in 1896 and it is this virus which has been utilized in most of the researches carried out in Europe and in this country.

During his travels in East Africa, in 1898, Koch presumably encountered this same disease which he considered to be identical with the Indian surra. He pointed out at the time the morphological differences which exist between this trypanosome and that of rats, and by means of a simple animal experiment he was able to differentiate sharply between the two organisms. Thus, in the blood of the rat the two organisms develop side by side and are quite easily distinguished by the aid of the microscope. If, however, the blood of such a rat is injected into a dog the nagana trypanosome alone appears in the latter and eventually causes death.

This work of Koch served as the immediate incentive for an exhaustive study of the rat trypanosome which was made in 1899 by Rabinowitsch and Kempner. To these workers much credit is due for introducing the staining methods which have thrown so much light upon the structure of these organisms. They were able to demonstrate many facts bearing upon the mode of multiplication, and in addition they showed that active immunity to *Tr. Lewisi* could be produced in rats. The study of the rat trypanosomes begun in 1900 by Laveran and Mesnil has led to the splendid series of researches on the pathogenic trypanosomes which have come from the Pasteur Institute since that time.

The *Tr. Lewisi* as found in the common rat is an excellent type of the whole group, and owing to the almost universal distribution of its host it has been found in all parts of the world. There is probably no city in which the rats are wholly free from this infection. This fact is of interest because it enables any one who is desirous of studying this organism to readily procure the needed material. It should be borne in

mind, however, that the infection is not necessarily evenly distributed throughout a community, but that it may be and is often localized in one or more places. Thus, at Ann Arbor, of the first 107 rats caught at different places and examined, five were found infected, and all of these came from the same barn. During the past six years we have repeatedly secured rats from this particular barn and invariably one or more in a catch have been found to harbor the parasite, whereas rats taken in other parts of the city have been relatively free from the infection.

It is an interesting fact that at times the white rat will be found to be spontaneously infected with *Tr. Lewisi*. The first observation on this point was made by Laveran and Mesnil in Paris, and later by Terry in Chicago. We have also repeatedly met with infected white rats purchased from dealers in the large cities.

Before taking up the more strictly pathogenic forms it will be desirable to consider briefly the structure and mode of multiplication of the rat trypanosome. In general, the facts ascertained by the study of this parasite hold true for the other forms. In the fresh blood film, when examined by the means of a medium objective, such as Leitz No. 7, the trypanosome readily attracts attention by the sudden commotion of the blood corpuscles in its immediate neighborhood. These are pushed aside or lashed about as the organism moves from place to place. The motion is fairly rapid, more so than is usually the case with the other blood trypanosomes. It will be seen that the body of the parasite is somewhat spindle-shaped and that one end terminates in a single long free whip or flagellum. The presence of this organ is an important characteristic and it is because of it that the trypanosomes are classed among the Flagellata. On careful observation it will be seen that the trypanosome usually moves with its flagellum foremost, picking its way among the corpuscles. The flagellar end is consequently spoken of as the anterior end. The opposite extremity or posterior end is rather sharply pointed, a fact which distinguishes it readily from the other trypanosomes.



FIG. 1.—*Trypanosoma Lewisii* in blood of rat. Note the sharp posterior end opposite the free flagellum; also nucleus and micro-nucleus. Magnification 1,500 times.



FIG. 2.—A multiplication rosette of *Tr. Lewisii* in blood of rat, showing division into eight cells and remnant with flagellum of original parent cell. The elongated micro-nucleus in each young cell shows two flagella, a long and a short one, indicating that further division is about to take place. Magnification 3,000 times.

On further inspection it may be possible to note the presence of an undulating membrane—a fin-like structure which extends from a point near the posterior end of the body to the base of the flagellum. This undulating membrane constitutes the real locomotive organ, the free flagellum probably having very little to do with the actual movement of the cell. This is seen in the fact that at least one blood trypanosome (*Tr. dimorphon*) is devoid or nearly so of a free flagellum. Moreover, in artificial culture many trypanosomes, although provided with a very long free flagellum, show scarcely any motion, owing to the rudimentary character of the undulating membrane.

The body of the trypanosome in the living condition is colorless or nearly so and the contents are nearly homogeneous; at most a fine granulation may be observed. Further details as to structure can only be made out by the application of suitable staining methods.

For this purpose the Romanowsky method in some of its numerous modifications is quite generally employed. No other process of staining brings out so well the structural characteristics of the trypanosomes and for that matter of all protozoa. The principle of the method consists in the use of a “ripened” or polychrome methylene blue which, with eosin, stains the nuclei and other nuclear structures a deep rose-red, whereas the plasma of the cell shows a pale to deep blue. In this way the details of the structure are brought out in sharp relief. The altered methylene blue owes its peculiar staining properties to the presence of at least two decomposition products, methylene azure (=di- and tri-methyl thionin) and methylene violet. The use of the former in the pure state is the basis of Giemsa’s modification, while the latter has been similarly utilized by MacNeal.

An examination of the illustration of the rat Trypanosome when stained (Fig. 1) shows the typical spindle form with the sharp posterior end. Near this extremity will be found a small but prominent roundish body known as the micro-nucleus, centrosome or blepharoplast. The function of this body appears to be that of a motile center. At all events, starting from the

blepharoplast is a prominent line which passes along one side of the parasite to the anterior end, at which it is prolonged as the free flagellum. Strictly speaking, the flagellum extends from the blepharoplast to the tip of the free portion. For about two-thirds of its length it is connected with the body of the parasite by means of a delicate membrane or fin-like structure known as the undulating membrane. It will be seen from this that the flagellum forms the back-bone, so to speak, of the undulating membrane. Under certain experimental conditions this delicate membranous connection may be dissolved away, in which case the flagellum, now freed for its full length, may continue to lash about vigorously for some time. The base of the flagellum does not originate immediately in the blepharoplast, but from a colorless or achromatic zone which surrounds this body.

Near the anterior end of the rat trypanosome will be seen a large oval or roundish body, which is the nucleus proper. In well-stained preparations the nucleus and flagellum are stained a deep red, the blepharoplast is dark red or almost black, whereas the plasma of the parasite is stained blue. The periplast or enveloping membrane may also take a red stain.

The length of the adult trypanosome, including the free flagellum, is about 25μ or about three and a half times the diameter of a red blood corpuscle. The ordinary width is about $1\frac{1}{2}\mu$. The divisional forms may of course be much longer and wider, while the young cells may be considerably smaller, and this is also true of the cultural types.

The stained preparations are especially useful in demonstrating the mode of multiplication of trypanosomes, which it may be said consists of a more or less unequal longitudinal division. Transverse division is wholly unknown. As might be expected, slight variations in the division process are observed in different species.

In the case of the rat trypanosome the cell which is about to divide becomes longer and wider, and at the same time the nucleus approaches the blepharoplast while the posterior end of the cell rounds up. The blepharoplast is usually the first

to show evidence of beginning multiplication. It elongates transversely and divides into two parts. To one of these the original flagellum remains attached, whereas the other half, as shown by MacNeal, gives rise to a new rudimentary flagellum, which then grows into the path of the former, probably because of least resistance at that place, and thus gives the impression that the flagellum is the first structure to divide. The division of the nucleus into two equal parts follows. Exceptionally the division of the nucleus may precede that of the blepharoplast, and in that case the cell may be seen to have three or even four nuclei, with but one blepharoplast. Such forms have been noted in tsetse-flies (Koch) and in cultures of *Tr. Lewisi*, etc. (Novy). Eventually the plasma of the cell undergoes fission resulting in the production of a young trypanosome, which may then detach itself from the larger or mother cell.

In addition to this direct division, the *Tr. Lewisi* may show during the first few days after infection has taken place, a modification of this process, which has been commonly designated as segmentation. The result is a group or rosette of 4, 8, or even 16 small trypanosomes with their flagella directed outward. The rosette formation is not essentially different from the simple direct division outlined above. It is rather the result of a delayed division of the protoplasm, due probably to the presence of anti-bodies, while the nuclei and blepharoplasts rapidly increase in number by consecutive binary division, thus giving rise to four, eight or more individuals. The successive stages can readily be followed in properly stained preparations. Thus, the parent cell rounds up at the posterior end while the nucleus and blepharoplast approach. The latter then divides, forming a rudimentary whip. At this stage the cell contains a single nucleus and two blepharoplasts, one having attached the original whip, the other having the new short one. At the next step the nucleus divides and the parent cell, which shows no evidence of division of the protoplasm, contains two nuclei and two blepharoplasts and two whips, one long and the other short. The two blepharoplasts in turn divide and the resulting cell still undivided will show two nuclei and four

blepharoplasts. The division of the nuclei soon follows and the body now contains four nuclei, four blepharoplasts, three of these having rudimentary flagella while the fourth still shows the original parent flagellum. Again the blepharoplasts divide and as a result the cell may show four nuclei and eight micronuclei. At the next stage the cell will show eight pairs of nuclear bodies, and about this time the common protoplasm divides, thus giving rise to eight young cells. The accompanying photograph (Fig. 2) taken by Dr. MacNeal shows such a rosette of eight cells, together with the remnant of the original mother cell. The rosette formation as described above is met with only during the early stages of infection. Brumpt has described a similar condition in the case of *Tr. Blanchardi* which he found in the garden dormouse. For some reason the really pathogenic trypanosomes do not occasion multiplication rosettes in the blood.

CONJUGATION.

Inasmuch as among the ordinary protozoa conjugation appears to be an essential condition for rejuvenation and perpetuation of the species, it would seem that a similar process must exist for the trypanosomes. The evidence on this point, however, is by no means as definite as might be desired. Schaudinn was the first to describe male, female and indifferent trypanosomes which he found in the stomach of mosquitoes that fed on owls infected with intracellular parasites. Pro-wazek more recently has described similar forms of *Tr. Lewisi* in the stomach of lice, and Keysselitz has done likewise for the trypanoplasma of fish as met with in the digestive canal of leeches. It must be confessed, however, that much work remains to be done before it can be said that the life-history of one of these organisms has been fully worked out.

PERSISTENCE OF TRYPANOSOMES.

In general, protozoal parasites tend to persist in the blood of the host for a long period of time even after a condition of immunity has been established. This fact is particularly seen in cattle which have recovered from Texas fever. Although

in the blood of such animals it is quite impossible to detect the piroplasmata which cause the disease, yet such blood, months and even several years after the attack, may convey the infection when injected into healthy cattle. Again, in malaria, as is well known, the plasmodium may remain in the blood for a considerable length of time. A similar condition to the above is met with in the case of trypanosomes. This is especially true for the so-called non-pathogenic forms found in rats, birds, fish, frogs, etc. In the case of the rat, the trypanosomes appear in the blood on about the fourth day after inoculation; they then rapidly multiply and reach their maximum about the tenth or the twelfth day, after which they decrease in number. It is not an uncommon occurrence to find them entirely absent at the end of two or three weeks. On the other hand, the parasite may persist in the blood, though in small numbers, for many months. For example, in one rat which we have had recently under examination, the parasites were constantly present for more than eleven months, at which time it accidentally died. In like manner we have observed the persistence of a trypanosome in a canary for about three months. The same is true for the flagellates of frogs, fish, etc.

The pathogenic forms may also persist in the blood of the host for a long period, and it is because of this fact that such animals, though apparently well, are likely to spread the disease. In the smaller experimental animals these trypanosomes may multiply with sufficient rapidity as to cause death in a few days, but in the larger ones, as the guinea pig, rabbit, horse and cow, and even in man, the disease may last for months and even years. Hence the necessity for promptly destroying infected animals, especially when these are brought into a non-infected territory in which the other conditions for transmission exist.

CULTIVATION OF TRYPANOSOMES.

The rat trypanosome was the first protozoon successfully cultivated in a pure form. Since 1903, when MacNeal and Novy reported their first results with *Tr. Lewisi*, a considerable number of other trypanosomes have been grown by them outside

of the body, notably *Tr. Brucei*, *Tr. Evansi*, various bird trypanosomes and those of mosquitoes. Thiroux has obtained like results with *Tr. paddæ* and *Tr. Duttoni*, while other workers have obtained partial results with a number of the pathogenic forms.

There appears to be a notable difference among the trypanosomes in the ease with which the first generation can be obtained. Little or no difficulty is encountered in starting a culture of *Tr. Lewisi*. This is especially true of the bird trypanosomes which can be grown with the greatest ease. Indeed, the detection of these organisms in birds is effected readily by the cultural method, although the microscopical examination is usually negative. The flagellates present in mosquitoes are likewise easily cultivated, the only difficulty is the likelihood of the cultures being overgrown by the bacteria which are invariably present in the stomach contents of these insects.

The cultivation of the more strictly pathogenic forms is somewhat more uncertain, but when the initial culture is once obtained the sub-cultures, it may be said, are assured. In other words, the adaptability of the trypanosomes to the artificial medium appears to be a variable one, but without doubt all of the trypanosomes, even the pathogenic ones, can be cultivated, although for some special conditions may be required.

As a rule little or no difficulty is experienced in carrying a culture through a large series of sub-cultures. For example, *Tr. Lewisi* has been kept under artificial cultivation for about two and a half years, during which time it passed through nearly 100 generations. In like manner the *Tr. Brucei* was carried through about 100 sub-cultures in about 15 months. Similarly, the bird trypanosomes have been kept in tube culture for over two years and a mosquito trypanosome has been maintained for about one year. Apparently there is no reason why these cultures can not be kept up, as in the case of bacteria, for an almost indefinite period.

The culture medium, in general, is a blood agar consisting of equal parts of defibrinated rabbit's blood and nutrient agar. The latter is melted and cooled to about 50°, after which the

rabbit blood is added and thoroughly mixed. The tubes, thus prepared, are allowed to set in an inclined position, after which they are at once inoculated. It is essential that the surface of the medium be moist and soft, and if this is not the case the tubes should be placed in an upright position for some minutes until some water of condensation accumulates at the bottom. After inoculation the tubes are closed with rubber caps and set aside either at room temperature or at 25°.

The initial culture usually requires a week or more, although not infrequently fairly rich growths may be obtained in three or four days. The sub-cultures when once adapted to the culture medium give an abundant growth in a few days and necessitate transplantation every seventh day. Unless this is done the cultures which are very prone to undergo involution changes may die out. With many of our cultures it has been found necessary, owing to the rapid growth at room temperature, to retard the multiplication by placing the tubes in a cool room.

On inspection the surface of the medium usually shows but little evidence of a growth. If, however, a loopful of the fluid from the surface is placed on a slide and examined under the microscope it will be found to be extremely rich in trypanosomes. At times, a thick, moist growth can be observed on the surface of the agar and when streak cultures are made, on blood agar plates, isolated colonies may be obtained. This fact has been utilized to effect a separation of trypanosomes from the accompanying bacteria.

The pure cultures are admirably adapted for the study of the life-history of an organism, inasmuch as the multiplication process can be followed under the microscope. The cultural forms as met with in the tubes differ in some important respects from the original blood forms. They are usually smaller, ranging from about 5 to 15 μ in length, although with some species a length of 50 to 75 μ or more may be observed. The blepharoplast is usually, though not in all cases, lateral or anterior to the nucleus, thus giving rise to the so-called *Herpetomonas* forms. This fact strongly indicates that the *Herpeto-*

monas as found in various insects is not a distinct genus but in all probability a mere multiplication form of a trypanosome. Moreover, some species, as for example, *Tr. avium*, show a distinct differentiation into two quite unlike forms which are suggestive of sexual types, male and female forms.

The trypanosomes which have been found in the stomachs of mosquitoes, tsetse-flies, house flies, etc., present essentially the same characteristics of growth as those which have been grown artificially. In other words they are to be regarded as cultural forms which develop in the alimentary tract in a manner analogous to those cultivated in the test-tube. From the evidence on hand it is more than probable that the genera *Herpetomonas* and *Crithidia* really represent cultural forms of true trypanosomes.

The cultivation of trypanosomes finds an immediate application in the differentiation of species. Many of the flagellated protozoa are much alike in shape and size and for that reason can hardly be distinguished with the aid of the microscope. The cultural forms, however, are so markedly different in the several species which we have studied as to make it very easy to recognize each kind. Thus, the *Tr. Lewisi* in culture is very unlike that of *Tr. Brucei* or of *Tr. avium*, and it is quite probable that fairly marked distinctions will be found for the trypanosomes of sleeping sickness, surra, etc. For this reason it is very desirable that persistent efforts be made to bring under cultivation all the important, more especially the pathogenic, trypanosomes. That this will in the end be realized, there can be no question.

PATHOGENICITY.

The rat trypanosomes are ordinarily considered to have no injurious action on the host. This is not strictly correct, for in severe infections of white and even wild rats there is evidence of fever and of marked depression. Occasionally, though very rarely, the infection may be so intense as to cause the death of young animals. Usually, however, the infected rats show little or no effect as the result of the presence of the parasite. The

continued presence of the organism for many months may be looked upon as the result of an immunization of the parasite against such anti-bodies as form or are present in the blood. A symbiotic condition may thus be established which may persist almost indefinitely.

It is an interesting fact which serves to distinguish *Tr. Lewisi* from all other trypanosomes that this organism cannot be transferred to any species of animal other than the rat. The injection of rat blood containing this trypanosome into white, gray or black rats results in an infection in about three or four days. Even large doses of such blood introduced into other animals are without effect except in the case of the guinea pig, where possibly a slight though transitory infection occurs. There are many other examples of trypanosomes having but a single definite host. On the other hand, the known pathogenic trypanosomes usually, as will be seen, are capable of infecting a large number of species of mammals.

TRANSMISSION.

The early studies on surra and especially on nagana demonstrated the rôle of certain biting flies as agents of transmission, and Rabinowitsch and Kempner, influenced by these observations, endeavored to find a similar mode of conveyance in the case of *Tr. Lewisi*. They were able to show that healthy rats developed the infection when placed in the same cage with the infected ones. Furthermore, they obtained positive infection by placing fleas from infected rats on healthy ones or by injecting the latter with suspensions of the crushed fleas. MacNeal was able to show in a similar way that the rat louse could convey the disease. In lice which have recently fed on infected animals the living trypanosomes can be readily detected in the ingested blood. Suspensions of such lice when injected into rats produce the disease, and, in one case, a healthy rat was infected by transferring to it a large number of lice taken from an infected rat.

It follows from the above that the infection is carried from rat to rat by fleas and lice, and this fact explains the localized

infections as often met with. Recently, Prowazek has endeavored to follow out the life-history of *Tr. Lewisi* in the body of the louse. In the stomach he observed "cultural" forms of a trypanosome and even described conjugation of male and female forms, but he was unable to infect rats by placing on them infected lice. On *a priori* grounds one might expect to find *Tr. Lewisi* to multiply in the gut of the louse as readily as in the test-tube, but the identity of such forms should be established.

Although it is not proven for all cases insect transmission appears to be the rule for the trypanosomiasis of mammals. There is good reason to believe that in the most important of these diseases, notably the tsetse-fly diseases of man and animals, the insect plays but a passive or mechanical part. In malaria transmission, on the other hand, the insect is an active host and not a mere vector of the virus. The infection of fish and amphibians, etc., likewise appears to be in relation with certain blood-sucking organisms, notably the leeches.

IMMUNITY.

It has already been pointed out that the *Tr. Lewisi* disappears from the blood after a variable length of time, depending upon the individual resistance of the rat. This usually occurs in from four to six weeks, although it may happen as early as two weeks and as late as a year. The rat which has once become free from the parasite can not be reinfected. In other words, it has acquired an active immunity. Rabinowitsch and Kempner were the first to show that the blood of such rats, after receiving a number of injections, possessed well marked preventive properties. In a dose of one c.c. the immune serum protected rats either when given before, simultaneously, or after inoculation with the trypanosome. A condition of passive immunity can hence be readily established.

The curative treatment of infected rats by means of such serum has given little or no result, undoubtedly because of its feeble activity. Similar efforts at causing the disappearance of *Tr. Lewisi* by injections of arsenite of sodium, human

serum, trypan-red, have been equally unsuccessful, although in several of the trypanosomiasis the experimental treatments with these agents have given fairly satisfactory results.

The production of active immunity in the case of the pathogenic trypanosomes is more difficult since with few exceptions the test animals usually succumb to the infection. In no case has it been possible to obtain an immune serum which could be used practically as a preventive much less as a curative agent. It has been possible, however, to immunize cattle, sheep, goats, etc., against several of these organisms, and such actively immunized animals have been utilized by Laveran and Mesnil as a means of differentiating trypanosomes. Thus, an animal immunized against *Tr. Brucei* remains susceptible to *Tr. Evansi* and to the other pathogenic forms. And *vice versa*, a cow immunized against surra will remain susceptible to nagana. In this way they have been able to establish a multiplicity of species which conclusion, however, Koch has questioned.

In a somewhat recent paper Koch has divided the best known trypanosomes into two groups, according as they presented constant or inconstant properties, especially with reference to their morphology, virulence and their behavior to the host.

In the first group he placed the rat trypanosome and that of galzielte for the reason that these are readily distinguished morphologically from the other pathogenic forms. Also, because in their virulence they show no variation. Notwithstanding consecutive passage through rats in the one case and through cattle in the other the virulence remains the same. Furthermore, their relation to their respective host is quite fixed since *Tr. Lewisi* can infect only rats and *Tr. Theileri* only cattle. Because of these facts he regards the organisms as fixed species which have acquired definite characteristics as the result of exclusive association with a special host in much the same way as the malarial organism in man. Obviously this group can be enlarged by the addition of a number of other trypanosomes having the same general properties.

In the second group he places the four more strictly pathogenic trypanosomes, those of surra, nagana, caderas and of

man. To these should be added the dourine and Gambian horse trypanosomes. He considers these organisms as lacking in definite distinguishing properties. Thus, morphologically, they are scarcely distinguishable among themselves; in virulence they show a very great variation and they are not limited to a single host. He interprets these facts to mean that the parasites have not become fully adapted to their hosts and hence have not developed into fixed species, but are rather in a state of mutation.

The views of Koch implying essentially a unity of species among the pathogenic trypanosomes can not be said to be established. The immunity reactions of Laveran and Mesnil and others cannot be accounted for satisfactorily by the assumption that these workers have studied one and the same trypanosome having merely a variable degree of virulence. Koch himself was inclined to regard the caderas trypanosome as distinct from that of nagana and of surra. Moreover, in a more recent publication, he himself has endeavored to differentiate the trypanosomes of the last two mentioned diseases by the "cultural" peculiarities of the flagellates found in the different species of tsetse-flies. The organisms found in the stomachs of these flies have been supposed to represent multiplication forms of the pathogenic trypanosomes, but this view, as we have shown elsewhere, is open to serious question. The conclusion which has been reached by most observers is to the effect that the several pathogenic trypanosomes represent distinct species, although each kind may be subject to considerable variation in virulence.

TRYPANOSOMES OF MAMMALS.

At the present time a large number of mammals have been shown to harbor in their blood, at times, parasites belonging to this group. Additional observations are being made from day to day and it almost seems as if there is scarcely a species of animal which may not show an infection of this kind. In the large majority of such animals the infection is of a mild type, indicating that the trypanosomes present are practically non-virulent. On the other hand, in a small number of animals,



FIG. 3.—*Trypanosoma Evansi* (Surra) from Mauritius, in blood of a mouse. Magnification 1,500 times.



FIG. 4.—*Trypanosoma Evansi* (Surra) from India, in blood of guinea-pig. Magnification 1,500 times.

trypanosomes are met with which are highly virulent and hence give rise to an almost invariably fatal disease. The great importance of this small group of trypanosomes will be readily recognized.

From the foregoing it will be seen that we may divide the mammalian trypanosomes into (1) non-pathogenic, and (2) pathogenic group. The non-pathogenic species are represented by the well-known rat trypanosome. Without going into any unnecessary detail it will be sufficient to state that similar parasites have been found in the field-mouse, mole, dormouse, house-mouse, hamster, bat, squirrel, gopher, guinea-pig, rabbit, monkey, etc.

The pathogenic trypanosomes require a special although necessarily a very brief description. They will be considered in the order as given in the following table:

Name	Discovered in	The Cause of
Tr. Evansi.....	1880	Surra.
Tr. Brucei	1894	Nagana.
Tr. equiperdum	1894	Dourine.
Tr. equinum	1901	Caderas.
Tr. dimorphon	1902	Gambian horse disease.
Tr. Theileri	1902	Galziekte.
Tr. gambiense	1901	Human trypanosomiasis or Sleeping Sickness.
Tr. ? (Piroplasma) Donovan.	1903	Kala-azar.

SURRA.

This term implying a rotten or diseased condition is a common designation in India for a disease of horses and camels. In the blood of the affected animals Evans found in 1880 an organism or "spirochæta" which he considered as the cause of the disease. It is interesting to note that the discovery of the malarial organism in man by Laveran and that of the first pathogenic trypanosome were made within a few weeks of each other. Since then the *Tr. Evansi*, as the organism is now known, has been repeatedly observed in outbreaks of the disease not only in India but elsewhere.

The disease is characterized by a remitting fever, consider-

able anemia and wasting, edema of the legs, belly and genitals, discharges from the eyes and nose, great muscular weakness and finally paralysis. Horses, mules, camels, dogs and cattle are particularly subject to the disease. As a result of the study of the disease in India it has been assumed that cattle usually recovered from an infection. While this apparently holds true for the Indian cattle it is not generally correct. Thus, during the Boer war the island of Mauritius became infected as a result of the importation of cattle from India, and as a consequence enormous losses were sustained among domestic cattle (25 to 30 per cent. dying) as well as among horses. Similarly, American cattle imported into the Philippines succumb to the disease.

At the present time surra is known to exist in many places outside of India. Thus, to the east it has been recognized in Burmah, Cochin-china, China, Philippines and in Java; on the west in Persia and on Mauritius. In Africa its presence has been established among the camels on the East Coast, also in Central Africa and in the Soudan. The diseases of camels known in different places as Mbori, Soumaya and El Debab, and the Algerian horse disease called mal de Zousfana, are probably all mild forms of surra. This fact has been clearly established by immunity experiments in the case of Mbori. Similarly, the *Tr. vivax* described by Ziemann in Cameroun is probably identical with the surra trypanosome.

Morphologically, the *Tr. Evansi* is so much like the *Tr. Brucei* as to be hardly distinguishable. Its length as given by Laveran and Mesnil is about 25μ , including the free flagellum, which is about 6μ long. The width is about 1.5μ . In general it is narrower and has a longer free whip than *Tr. Brucei* and is more actively motile. Like the other pathogenic trypanosomes it is characterized by a well-developed prominent undulating membrane (Figs. 3 and 4).

The artificial culture will probably afford the surest means of distinguishing it from the closely related nagana trypanosome. Thus, the Philippine surra organism has been grown on blood agar for a period of sixty-five days. Although all efforts at transplantation failed there was no question but

that multiplication had taken place within the tubes. The cultural forms present were entirely different from those of *Tr. Brucei* (Novy, MacNeal and Hare). Laveran and Mesnil were likewise able to keep it alive for three months, into the second generation, but further efforts at transplantation failed, as did also the animal inoculations. Thomas and Breinl obtained similar results, the organism remaining alive for thirty-seven days. In view of the variations shown by the surra of different countries it is very desirable that attention be given to the cultural characteristics of the divers strains of *Tr. Evansi*. In this way it will be possible to pass upon the question of the identity or non-identity of the various forms of so-called surra.

TRANSMISSION.

It is noteworthy that biting flies were credited with the transmission of the disease by the natives of India and that this view was accepted by Evans and others long before the rôle of insects was recognized in Texas fever, malaria, etc. It is only quite recently, however, that the question has been submitted to an experimental test.

The tsetse-fly which plays so important a part in the spread of the African trypanosomiasis is not known in Asia and hence other genera of biting flies must be considered. These are represented more especially by the *Stomoxys calcitrans* and *Tabanus tropicus*. Several investigators have found trypanosomes in the alimentary tract of flies which have fed on infected horses, and Rogers, Musgrave and Clegg and others have conclusively shown that infected flies will transmit the disease provided they are allowed to bite clean animals within a few hours after the infective feed. It is evident, therefore, that several species of flies may serve as transmitters of the virus, probably as mere mechanical agents. The possibility of transmission by other insects cannot be excluded for the present. The infection of dogs is usually ascribed to their eating the carcasses of animals which have succumbed of surra, the virus probably entering through abrasions in the mouth or alimentary canal, though obviously fleas and flies may also take part in the transmission.

NAGANA.

The early travelers in South Africa, among these Livingstone, encountered a disease of domestic animals which they designated as the Tsetse-fly disease or the fly disease for short, this name being given on the supposition that it was caused by the bite of the tsetse-fly, *Glossina morsitans*. The term nagana, which has come into use since Bruce made his studies of this disease, implies in Zulu "to be low or depressed in spirits."

The disease has evidently existed in Zululand from the earliest times. It is not, however, confined to that region but has been met with on the west coast as far as Senegambia and on the east as far as the Red Sea. Like the surra of India it affects the horse, mule, donkey, ox, dog, cat and many of the wild animals. It is said to be invariably fatal to the horse, donkey and dog, while but a small per cent. of the cattle recover, in which respect it has been supposed to differ from the surra of cattle in India where recovery was apparently the rule. Singularly enough, while nearly all mammals are subject to either natural or experimental infection, man appears to be perfectly immune.

The duration of the disease varies greatly, not only among the different species of animals but also among animals of the same species. Thus death may occur in from one to two weeks or as late as two to three months. Cattle may likewise die early, though the disease may be protracted for a year or more. Occasionally they may recover as in the case of the experimental inoculation of Brittany cattle by Nocard. In sheep and goats the course of the disease is also a chronic one and may last for two to six months and recovery may take place as has been noted by Laveran and Mesnil.

The animals which recover from the infection acquire an active immunity, as is seen in the fact that they cannot be reinfected with the same virus. Repeated injections serve to hyperimmunize the animals and the serum of such possesses a slight preventive action. The animals immunized to nagana



FIG. 5.—*Trypanosoma Brucei* (Nagana or Tsetse-fly disease) in blood of rat. One of the cells is in process of division. Magnification 1,500 times.



FIG. 6.—*Trypanosoma equiperdum* (Dourine or Mal du coit) in blood of mouse. One of the cells is in process of division. Magnification 1,500 times.



FIG. 7.—*Trypanosoma equinum* (Caderas) in blood of mouse. Note the apparent absence of blepharoplasts or micro-nuclei. The double flagellum indicates divisional changes. Magnification 1,500 times.



FIG. 8.—*Trypanosoma dimorphon* (Gambian horse disease) in blood of mouse. One cell is dividing. Note the absence of a free flagellum. Magnification 1,500 times.

remain susceptible to infection with the other trypanosomes (as has been shown by Laveran and Mesnil, also by Nocard and Vallée), and consequently this reaction may be interpreted as demonstrating that nagana is specifically different from surra and the other trypanosomiasis.

In the large animals the affection is characterized by fever, anemia, edemas of the extremities and abdomen, and extreme emaciation. The animal may waste to a mere skeleton and often becomes blind. Its appetite may last until the end.

In the blood of the diseased animals Bruce discovered in 1894 the trypanosome which now bears his name. As has been pointed out heretofore, this organism is scarcely to be distinguished either morphologically or biologically from the *Tr. Evansi* (Fig. 5). The immunity experiments referred to above have served to show that the diseases are distinct entities.

The *Tr. Brucei* was the first pathogenic trypanosome successfully cultivated (Novy and MacNeal). Some difficulty is experienced in securing the first generation, but after that there is none. It has been possible to carry such a culture through nearly 100 generations at room temperature and at 25°. When once thoroughly adapted to the blood agar medium a very rich growth can be obtained in from three to four days. Even after cultivation for two years such cultures are capable of infecting guinea-pigs. It has not been possible as yet to obtain non-virulent cultures which could be used for the purpose of vaccinating animals against the virulent type.

The cultural characteristics are very marked and serve to differentiate it from *Tr. Lewisi* on the one hand and *Tr. Evansi* on the other. In view of the fact that nagana of some parts of Africa differs to a greater or less extent from that of Zululand, which fact has suggested the existence of a number of varieties or even a group of allied diseases, it is desirable that in such cases the cultural characteristics of the organism be ascertained.

TRANSMISSION.

The general impression regarding the rôle of the tsetse-flies in the transmission of nagana was fully established by the

splendid investigation of Bruce. This was executed in Zululand, on the top of Ubombo, at an elevation of about 1900 feet. Although this hill was in the midst of the fly-country, Ubombo itself remained free from tsetses from the beginning of the investigation in 1894 until 1897, at which time nagana for some reason spread beyond its former boundaries, and even reached the top of the hills. Bruce early noted the remarkable fact that no cases of the spontaneous disease occurred on Ubombo hill, notwithstanding the constant and close association of healthy horses, cattle and dogs with those suffering from the disease and the presence of several species of blood-sucking flies other than tsetses. This observation was confirmed later by Martini who found two infected Togo ponies in the Berlin Zoological Garden, and yet in spite of the presence of ordinary biting flies the infection did not spread among the other animals. These facts go to show that nagana is not spread by ordinary biting insects such as *Stomoxys*, mosquitoes and fleas.

Bruce further showed that horses when taken for a few hours into the fly country—even though they were not allowed to eat or drink while there—contracted the disease, thus showing that the latter was not conveyed by the food or drink but in all probability by the bites of the flies.

It was further proved that tsetses brought to Ubombo and kept for several days could then bite dogs without producing the disease. Infection, however, did occur if they were allowed to feed on dogs immediately after feeding on an infected animal. This result was also obtained if the flies were allowed to bite at 12, 24 and 48, but not at 72 hours after the infective feed.

Bruce examined the proboscis of the flies at varying hourly intervals after they had fed and found rarely more than a single trypanosome. In the stomachs of such flies the trypanosomes were usually present as long as the blood remained. Up to 55 hours they were always present, but none were found on the sixth day. Owing to the presence of the trypanosomes in the stomachs it might be expected that such flies, if minced up and injected into a dog, would produce the disease. This, however, Bruce was unable to accomplish except in one case

where the fly had fed but half an hour before on an infected animal. This result is difficult to explain, especially since we have been able to infect mice by injecting mosquitoes which had an infective feed 24 to 36 hours before.

Within the past year it has been shown by Koch that several species of tsetse-flies contain trypanosomes in their stomachs, at the time of capture even when there is no evidence of the presence of blood to indicate that the flies had recently fed on an animal. It was supposed by him that the flagellates found in the stomachs of the flies represented developmental forms of *Tr. Brucci*, although he was unable to infect flies by feeding on infected animals, and moreover failed to infect rats by injecting the contents of the stomachs of such flies. As will be seen similar observations have been made by Gray and Tulloch with reference to the trypanosome of sleeping sickness. The facts noted, however, are all open to an entirely different interpretation, and it is more than likely that the organisms seen in the flies represent, as we have shown elsewhere, a harmless intestinal parasite much the same as that found in mosquitoes and other insects.

Notwithstanding the above, the fact remains that the tsetse-flies can and do convey the disease provided that they bite within a few hours or at most a day or two after they have fed on a diseased animal. The fly, therefore, acts as a mere carrier of the trypanosome, and that being the case it was necessary to establish the natural source of the disease. It had been shown previously by Lingard in India and confirmed later by Musgrave in Manila that rats may harbor the surra trypanosome. Bruce was led, therefore, to believe that a similar source might be found in the big game of the fly-country, especially as the general impression prevailed that where there was no wild game there was no nagana. Accordingly, he examined the blood of various animals shot in the fly-country, but could find no trypanosomes in their blood. Subsequently, however, he did find the adult organism in three wild animals. Acting on the supposition that the parasites might be present in such small numbers as to escape micro-

scopical detection, he injected the blood into dogs, with the result that in the first series nine out of thirty-five dogs thus inoculated developed nagana.

In brief, it has been shown that the large game harbors the parasite without any injurious effect in much the same way that the common rat carries *Tr. Lewisi*. In such animals the disease is probably chronic and non-fatal and hence they serve as a reservoir for the virus which they supply to the tsetses. It has since been shown that with the extermination of the large game in some parts of South Africa, due to the introduction of rinderpest, the fly disease has become a negligible quantity.

Although considerable effort has been expended to discover a preventive and curative treatment for this infection, the results thus far have not been very satisfactory. The administration of arsenic is of no preventive value but in infected animals serves to prolong life. When given in sufficient dose to the small animals it causes the trypanosomes to disappear from the blood, but only temporarily. After a few days they reappear, and on repeating the injection of arsenic they vanish as before. By repeating the treatment it has been possible to keep rats alive for 79 days, whereas the controls die in about 5 days. Laveran and Mesnil were unable to effect any cure with arsenite of sodium employed in this manner. Thomas has obtained much better results with a compound of arsenic and anilin known as atoxyl. With this he has been able to cure infected rats, guinea-pigs and a rabbit.

The trypan-red introduced by Ehrlich and Shiga likewise causes a temporary disappearance of the trypanosomes, but it only exceptionally is able to do more than prolong the duration of the disease. A number of other anilin dyes have been used, some of which have given very encouraging results. The recent studies of Mesnil and Nicolle on the "benzidine colors" are very promising, and it is to be hoped that an efficient "chromotherapy" will be discovered.

The alternate treatment with trypan-red and atoxyl has given fair results, and it is quite possible that some modification of this procedure may prove serviceable in practice.

The action of human serum as a curative agent is of especial interest. It may be assumed as established that man is not subject to infection with *Tr. Brucei*. At all events, though man is attacked in the fly-country by the tsetses, no ill effects have been noticed from such bites. The normal sera of diverse animals have been tested by Laveran as to their effect on the trypanosome and of such only the human serum was found to be efficacious. It not only can prolong the duration of the disease but is capable of curing mice. An alternate treatment with arsenic and serum has given still better results.

From the brief outline given above it will be seen that arsenic, certain anilin dyes and human serum are the only agents known at present which can influence the course of the infection with *Tr. Brucei*. A practical preventive and curative treatment, however, has not yet been discovered, and as it is obviously impossible to reach and destroy all infected animals the prevention of nagana remains a serious problem.

DOURINE.

This disease, unlike surra and nagana, is not limited to the tropical countries, for in the past it has been met with in many parts of Europe and it is even said to occur in the United States. Such reports, however, are based purely upon circumstantial and clinical evidence, for up to the present time no one has demonstrated in this country the presence of the specific organism in such cases. The disease it should be stated is especially prevalent along the Mediterranean littoral.

The trypanosome of Dourine, *Tr. equiperdum*, was discovered in 1894, the same year as *Tr. Brucei*, by Rouget, a French army veterinarian. By successive passages through rabbits he was able to keep the organism, derived from a stallion in Algeria, for over two years. Since then other observers have confirmed and extended his work.

One notable feature of the disease is that it is not spread, so far as is known, by flying insects. In this respect it differs therefore from surra and nagana and the other trypanosomiasis.

The evidence on hand goes to show that it is spread exclusively by sexual contact, hence the designation *Mal du coït*. It is restricted to breeding equines, and on account of the peculiar skin lesions it is sometimes spoken of as horse syphilis.

The usual chronic form of the disease is characterized by edema of the genitals, a moderate fever and slow wasting. In from one to two months, plaques of varying size appear on different parts of the skin. The wasting becomes more pronounced and paralysis of the hind quarters becomes manifest. In the last stages of the disease the anemia is profound, the animal becomes paralyzed and is unable to rise. The infected animals may die in from two to ten months, exceptionally they live for as long as two years.

Experimentally, the disease is readily reproduced in the horse, ass, dog and rabbit. Rats, mice and guinea-pigs are apparently refractory to the fresh virus, but after repeated passage the latter will prove infective. It is an interesting fact, first established by Rouget, that a lesion of the mucous membrane is not necessary in order that infection shall result. The direct application of the virus to the conjunctiva or to the vagina is sufficient to cause an infection. The disease has not been produced by ingestion of the parasite.

Although very scarce, the trypanosome can be detected in the blood of the infected animal and especially in the freshly formed plaques. Morphologically, it can hardly be distinguished from the other pathogenic trypanosomes (Fig. 6). The most important difference according to Laveran and Mesnil consists in the absence of the protoplasmic granules such as are present in *Tr. Brucei*. Thomas and Breinl were able to keep the organism alive on blood agar for 17 days, at which time it was still infective, but no sub-cultures could be obtained.

Animals such as dogs which have recovered from the disease possess an active immunity. But when inoculated with caderas (Lignières), or nagana (Nocard), they promptly succumb to these diseases, thus showing that dourine is specifically different.

The treatment of dourine is on the whole as unsatisfactory as that of the other trypanosomiasis. Arsenic, trypan-red and

human serum have an action similar to that noted in connection with nagana.

CADERAS.

The disease known as *Mal de caderas* is widely prevalent in many parts of South America from the Amazon on the north to Bolivia on the south. It is the only trypanosomatic disease which has been recognized on the American Continent. It has been the subject of repeated studies, but its nature was not recognized until 1901 when Elmassian at Assumption discovered the trypanosome which Voges designated as *Tr. equinum*. The name given above refers to the characteristic symptom of the disease, the paralysis of the hip or hind quarters.

Caderas occurs almost exclusively among horses, although instances of spontaneous infection of dogs are known. The ass and mule are more resistant than the horse. The disease may be of short duration lasting from a few weeks to one or two months, or it may be of a very chronic form which persists for many months.

It is attended with a marked remitting fever; the animal rapidly loses weight, although the appetite continues. Eventually the hind quarters begin to drag and finally complete paralysis results. Anemia and albuminuria are common, and hematuria may be present. A notable feature is the almost complete absence of edemas which are almost always present in nagana, surra, etc. The trypanosomes are not very numerous in the blood and often cannot be detected microscopically. The inoculation of such blood into susceptible animals will result in infection.

Rats and mice are particularly susceptible, and in these the trypanosomes become extremely rich and death occurs in from one to two weeks after inoculation. Most of the smaller mammals are likewise susceptible, but the disease is of longer duration. Goats, sheep, cattle and hogs develop a very mild infection without any visible manifestation. The organism is present in the blood in such very small numbers that it can

scarcely be detected except by inoculation of the blood into mice. By this means it has been shown that the blood may harbor the trypanosome for from two to six months. With these animals recovery is the rule, and they become immune.

The *Tr. equinum*, although it is of the same form and size as the other pathogenic trypanosomes, is nevertheless readily differentiated from these by one important characteristic and that is the apparent absence of the micro-nucleus or blepharoplast. This structure is so inconspicuous that its existence has been denied by some (Fig. 7).

Attempts at cultivation have been made by Rabinowitsch and Kempner, and Laveran and Mesnil but without any definite success. Thomas and Breinl observed an apparent multiplication in a blood agar tube, 29 days old, and with this successfully infected a rat. Sub-cultures were not obtained.

As to the mode of transmission of caderas very little is known at present. The conveyance by insects is by no means established, although it has been possible to infect horses by *Stomoxys* which had previously fed on infected animals. On the other hand, healthy and infected animals have been kept together or at most separated by a fence without the infection spreading.

The results of treatment in caderas have on the whole been more favorable than in the case of the other trypanosomiasis. Thomas and Breinl obtained with atoxyl cures in rabbit, guinea-pig and rat. Ehrlich and Shiga were able to cure mice by means of their trypan-red. Moreover, previous inoculations with the dye served to prevent infection. Several other dyes are now known which in a single injection will cure infected mice (Mesnil and Nicolle). Human serum has been shown to be as active against caderas as against nagana. As with the latter, the disease is prolonged in the infected mice and exceptionally a cure is effected.

GAMBIAN HORSE DISEASE.

This disease was first recognized by Dutton and Todd, in 1902, among the horses of Senegambia. Of 36 examined 10

were found to have the trypanosome in their blood. As far as known no other domestic animal is subject to the disease, although most mammals, including sheep, goats and cattle, can be infected.

The natural disease is very chronic in character and differs from nagana by the absence of edemas. In the latter respect it agrees with caderas, but as will be seen the trypanosomes of these two diseases are easily differentiated. The duration of the disease is not known, though it probably lasts for a few months to more than a year. In an experimental infection of a horse, Laveran and Mesnil noted the formation of an edematous patch, but otherwise the animal did not appear to be ill. There was an occasional rise in temperature and trypanosomes were present at first in the blood, but later were recognized only by inoculation of rats and mice. In this way they were found to be present as late as the one-hundred-and-eighty-third day. The disease is presumably transmitted by biting flies, although no positive evidence on this point has been obtained.

The examination of living and stained preparations shows the presence of two forms, hence the name of the organism, *Tr. dimorphon*. The short form is about 12μ while the long one is 20 to 25μ . A similar occurrence of long and short forms has been noted in galzielte and in bird infections. Unlike as in *Tr. Brucei*, the undulating membrane is not conspicuous but by far the most important characteristic is the absence of a free flagellum. This condition is due to the prolongation of the protoplasm of the cell along the flagellum to the very tip. This feature serves to identify the organism in the same way that the minute blepharoplast characterizes the trypanosome of caderas.

The artificial culture of this organism was attempted by Laveran and Mesnil, but although they succeeded in keeping it alive on artificial media for over a month they were unable to secure sub-cultures. Thomas and Breinl were more successful, for they maintained it on blood agar for 78 days and were able to infect animals as late as the twenty-third day.

The structural peculiarity of the trypanosome serves at once to differentiate the infection from all other flagellate diseases. As further evidence of its individuality it may be mentioned that animals immunized to the other trypanosomes remain susceptible to inoculation with *Tr. dimorphon*. Thus, goats which have been vaccinated against surra, nagana and caderas, are very sensitive to this parasite.

The treatment of the experimental infections has not been as good as with nagana and the other trypanosomiasis. Thus, arsenic, either in the form of arsenite of sodium or atoxyl, causes the organisms to disappear temporarily and the duration has been prolonged, but no cure has been effected. Trypan-red has a similar action on the trypanosomes, but neither Laveran and Mesnil nor Thomas and Breinl have noted any definite curative powers. Human serum in large enough dose may also cause the disappearance of the trypanosomes for a varying length of time. The action, however, is more feeble than in the case of nagana.

GALZIEKTE.

A trypanosomiasis of cattle wholly distinct from nagana or surra appears to exist throughout South Africa and probably it occurs elsewhere. Thus, a similar if not identical infection has been observed in East Africa (Sander, Panse), in West Africa (Schilling, Ziemann), in the Trans-Caucasus (Ziemann), and in India (Lingard). The disease itself has been known for many years and is known by a variety of names, such as gall-sickness or galziente, malaria, jaundice or bilious fever of cattle.

Compared with the other diseases of domestic animals in Africa this is of but slight importance. The disease is marked by a light fever, which lasts several days, and a severe anemia, which may be either acute or chronic. The mortality as given by Theiler is but 12.5 per cent.

In the blood of the infected cattle in 1902 Theiler discovered an unusually large trypanosome which Laveran and Bruce, independently, designated as *Tr. Theileri*. It is the largest

of the pathogenic trypanosomes and is about the size of the large form of *Tr. avium* as met with in robins and blue-jays. Like the *Tr. avium* and *Tr. dimorphon* it occurs in the blood in two forms, one short and the other long. The former are 25 to 30 μ in length by 2 to 3 μ in width; while the latter may be 60 to 70 μ long and 4 to 5 μ wide. It is very actively motile and has a prominent undulating membrane and a long free flagellum.

Experimentally the disease can be readily transmitted to cattle by injection of the infected blood. The trypanosomes may be very numerous or on the other hand quite scarce. Like the trypanosome of the rat the *Tr. Theileri* appears to be limited to a single host, since all attempts to inoculate other animals have failed. After recovery the cattle are immune. The disease appears to be transmitted by the bite of a fly, *Hippobosca rufipes*.

HUMAN TRYPANOSOMIASIS.

(Sleeping Sickness.)

For a long time it was supposed that man was not subject to trypanosomatic infection. This appears to be true for surra and nagana as well as for the other diseases discussed above. Certain it is that the bites of insect carriers are without effect in man, and even accidental inoculations have occurred without any observable result. In a way, this immunity of man to the animal infections is largely if not wholly due to the peculiar action of human serum on these trypanosomes.

The first authentic case of human infection with trypanosomes was observed at Bathurst, Gambia, in 1901, by Forde, who, however, did not recognize the nature of the organisms which he described as "small, worm-like, extremely active bodies." On subsequent examination of the patient, who was an Englishman in the government service, in December, 1901, by Dutton, the organism was at once recognized as a trypanosome and was named by him *Tr. gambiense*. On his return to England, Dutton examined 115 blood films obtained from native

children in Gambia and one of these preparations showed a double infection with malarial parasites and trypanosomes. The result of these discoveries was of far-reaching importance. The Liverpool School of Tropical Medicine at once sent out an expedition consisting of Dutton and Todd to Senegambia with the result that these observers recognized seven cases out of 1043 persons examined. About the same time (1902) Manson diagnosed the disease in a woman, the wife of a Congo missionary. Other cases were soon reported by Broden at Leopoldville, Brumpt at Boumba, and by Kermorgant. The existence of a human trypanosomatic fever was thus established but its relation to the terrible disease known as Sleeping Sickness was not suspected.

Sleeping sickness itself has been known to exist on the West Coast of Africa for more than a century. From the time of Winterbottom, who described it among the slaves of Benin in 1803, it has repeatedly been studied by English and French physicians and missionaries on the Gold Coast and at Sierra Leone. Although undoubtedly hundreds of slaves infected with the disease were transported to the West Indies, there is no reason to believe that new cases ever developed on this side of the Atlantic.

The disease is stated to exist on the West Coast of Africa from Senegal on the north to Benguella in Angola on the south. In certain localities of this vast territory it has proved particularly destructive, notably along the Lower Congo. Since the establishment of the Congo Free State in 1885 the disease has been carried to the Upper Congo in the first place by traders, and secondly by military expeditions (1892-1896) against the Arab raiders. In 1901 its presence was reported for the first time in Uganda, where already it had caused an enormous destruction of life. This serious outbreak is generally supposed to be due to the return of the remnants of Emin Pasha's army which were brought from the regions west of Albert Nyanza, during the years 1892-95, and established on Victoria Nyanza in Busoga. Whatever its origin, the disease has since its introduction spread along the entire north shore of Lake Victoria

Nyanza and has even passed down the Victoria Nile as far as Wadelai (Greig).

The British and Portuguese governments, recognizing the need of definite information regarding the cause and spread of sleeping sickness, appointed commissions to investigate the disease. Considerable attention was given at first to the supposed bacterial cause, and it was while engaged in this study that Castellani in Uganda noted the presence of trypanosomes (Nov., 1902), in the cerebro-spinal fluid of five cases of sleeping sickness. At the time he did not consider that this trypanosome had any causal relationship to the disease, but later, on the suggestion and with the aid of Colonel Bruce and others, he examined additional cases of the disease and was able to report the presence of trypanosomes in 70 per cent. of the cases (April, 1903). Subsequent studies by Bruce, Nabarro, Greig, and others have demonstrated the constant occurrence of the *Tr. ugandense* (Castellani) in either the blood or cerebro-spinal fluid of sleeping sickness cases.

The trypanosome found in sleeping sickness was at first supposed to be distinct from the *Tr. gambiense* of Dutton, but subsequent researches have shown that in all probability the two organisms are identical and that the trypanosomatic fever is but the first, while sleeping sickness is the last, stage of the human disease.

Still more recently the important fact has been brought out that glandular enlargements are a constant feature of early cases of human trypanosomiasis; in other words, that sleeping sickness during the early stage is a specific polyadenitis caused by the *Tr. gambiense* (Greig and Gray). It has been shown that the trypanosome could practically be always found in such enlarged glands, and Dutton and Todd have pointed out that cervical gland palpation is a simple and very accurate method of detecting cases of trypanosomiasis in which clinical signs are wanting. The recognition of the existence of such cases explains the ease with which the disease has been carried into uninfected districts by the migration of apparently healthy persons. And furthermore, being a simple means of diagnosis

of the earliest stage of the infection, it enables putting into effect measures for the prevention of the disease. This means the exclusion and removal of persons having glandular enlargements from uninfected territory and their segregation as far as possible. The necessity of adopting every possible means of arresting the progress of the disease is seen in the fact that in many villages Dutton and Todd found from 30 to 50 per cent. of the population infected; which means, since the disease so far as known is invariably fatal, that at least a third of the people in such districts will probably die of trypanosomiasis. That this is far from exaggerating the conditions of things is evidenced by the history of the spread of the disease in Uganda, where in a few years hundreds of thousands have died of the infection and whole regions have been depopulated.

It has been shown conclusively that sleeping sickness is conveyed by the bite of a tsetse-fly, *Glossina palpalis*. This species is different from that which carries the nagana of South Africa. Whether other species of this genus can convey the disease has not been established. In all probability, as in nagana, the fly is a mere vector, a mere mechanical means of carrying the trypanosome from the sick to the healthy persons. The presence of multiplication forms of trypanosomes in the stomachs of such flies (Gray and Tulloch, Koch) has been taken to show that the fly is not a passive carrier of the organism. The tsetse trypanosome, however, has not been shown to be identical with the human parasite—in fact, there is reason to believe that they are in no wise related, and that the former (*Tr. Grayi*, Novy) is a harmless parasite peculiar to the fly.

Human trypanosomiasis is characterized by two stages. In the first the trypanosomes exist in the blood but always in small numbers. An irregular remitting fever is the chief symptom of this stage. The pulse and respiration are accelerated. Slight edemas and erythemas are at times met with and in addition enlarged glands and spleen. Owing to the mildness of these symptoms the disease passes unnoticed among the natives. The second stage follows after the lapse of a variable length of time. It is this stage which is known as



FIG. 9.—*Trypanosoma gambiense* (human trypanosomiasis or sleeping sickness) in blood of a rat. Two types are shown; the broad pale form (female ?) is dividing. Magnification 1,500 times. MacNeal's stain.



FIG. 10.—*Trypanosoma gambiense* from same preparation as preceding, showing the usual form, some of the cells in process of division. Magnification 1,500 times.

sleeping sickness. The fever is marked especially toward evening. The patients become dull and apathetic and complain of intense headache. Weakness of the arms and legs develops, speech becomes difficult, and emaciation sets in. Somnolence increases and a comatose condition supervenes with death.

The *Tr. gambiense* is present in but small numbers in the blood of man. Most of the experimental animals are subject to infection and in the blood of such it may become very numerous. The course of the disease in monkeys is at times very suggestive of that in man. The baboon has been supposed to be refractory but Thomas and Breinl have succeeded in infecting four of these animals. The strain isolated from one of these proved to be highly virulent. The macaques are quite susceptible, and die in from one to two months. In the horse and donkey the infection is very chronic, with very few parasites in the blood, and recovery seems to occur. The cow is even more refractory than the horse. Sheep contract also a mild infection and recover. Goats are apparently more susceptible and death may result (Thomas and Breinl). Dogs are easily susceptible but may survive for six or even nine months, though death may occur in from five to six weeks. Cats are likewise subject to infection and especially kittens, in which the parasites appear in large numbers and cause death in from three to seven weeks. In rabbits and guinea-pigs the trypanosomes are very scanty, especially in rabbits, and the infection is very chronic, lasting for several months. In mice the infection is also very slight and recovery may take place. On the other hand white rats are quite easily infected. As a result of intraperitoneal injection we have seen the parasites appear in the blood within three days, though the period of incubation is usually given as about fifteen days. The parasites may increase enormously in numbers for a while and then almost completely disappear from the circulation (Figs. 9 and 10). Death occurs in from one to three months.

Morphologically the *Tr. gambiense* resembles very closely *Tr. Brucei* and *Tr. Evansi*. The cultivation of the organism has been attempted by Laveran and Mesnil and also by Thomas and

Breinl. The former were able to keep it alive on blood agar for nineteen days but were unable to obtain sub-cultures or to infect rats with such material. The latter succeeded in maintaining it for sixty-eight days but they also failed to obtain actual sub-cultures.

Normal human serum which possesses a pronounced action on the trypanosomes of caderas and nagana is without effect on *Tr. gambiense*. According to Thiroux the serum of cases of sleeping sickness in which the blood is free from trypanosomes possesses a slight protective action with respect to mice.

The treatment of experimental animals with arsenic (atoxyl), trypan-red and other anilin dyes (Mesnil and Nicolle) has given very encouraging results. With the aid of an alternate treatment with arsenic and trypan-red Laveran was able to cure monkeys. With the exception of one case in a woman, reported by Dutton and Todd, the treatment of the human cases has thus far been ineffectual.

KALA-AZAR.

A brief consideration must be given at this place to a peculiar organism which is present in Kala-azar and the cachexial fever of India, especially since recent studies go to show that it is a flagellate and closely related to the trypanosomes. In 1903 Leishman found certain bodies in the spleen of a fatal case of the disease and surmised that they were degenerated forms of trypanosomes. Very shortly after Donovan at Madras confirmed this finding but he was unable to get any trace of trypanosomes. Laveran, to whom specimens were submitted, pronounced the parasite to be a Piroplasma and gave to it the name *Piroplasma Donovanii*. Ross regarded it as representing a new genus and for that reason he called it *Leishmania Donovanii*.

The investigations of Rogers, which have since been confirmed by others, have thrown much light upon the nature of this organism. He found that when the fresh blood, obtained by spleen puncture, was transferred to test-tubes containing a few drops of 2 to 5 per cent. citrate of soda in normal salt solution, the parasites remained alive for many days, and after

about three days some of them developed into elongated flagellated bodies which he took to be trypanosomes, although no undulating membrane could be detected. Rogers has since found that the flagellation took place more uniformly and regularly if the citrated spleen blood was faintly acidified with citric acid. The flagellated forms develop best at about 22°, the same as in the case of the cultural trypanosomes. The Leishman-Donovan bodies it may be said resemble greatly rounded up forms of trypanosomes. They show a nucleus and a micro-nucleus. In the citrated blood these forms increase in size, elongate, and give off a flagellum. The latter starts from the blepharoplast which lies close to the anterior end of the cell. It is perhaps on account of this close proximity to the end that, as is the case of the mosquito trypanosomes, no undulating membrane can be made out. At all events, on account of the absence of this structure, Rogers has recently come to the conclusion that the organism belongs to the herpetomonas group and not to the trypanosomes, and he has designated it as the *Herpetomonas*¹ of Kala-azar.

The fact that we have in this case an undoubted flagellate developing from the Leishman-Donovan bodies goes to establish a certain relationship between this disease and those which have been heretofore considered. The exact position of the organism can only be determined by further study. It certainly presents some of the cultural characteristics of the bird trypanosomes and more especially of the mosquito flagellates. It may be added that, while ordinarily the mosquito herpetomonas fails to show an undulating membrane, in some cultures evidence of such can be observed, and hence the apparent absence of this structure does not necessarily exclude the organism from the group of trypanosomes.

As to the transmission of this fatal disease nothing definite can be stated. Rogers is of the belief that the common bed-bug or possibly mosquitoes are the most likely hosts. By allow-

¹ Through typographical error this genus is given as *Hepatomonas* in Rogers' paper.

ing mosquitoes to bite a patient Patton has been able to find in their stomachs flagellates, *herpetomonas* and *crithidia*, such as we have described in these insects, and consequently these forms cannot be considered as stages of this parasite. From a private communication it appears that Patton has succeeded in finding developmental forms of the parasite in the common bed-bug.

TRYPANOSOMES OF OTHER ANIMALS.

In the foregoing an effort was made to give a brief résumé of the mammalian trypanosomes, particularly of the pathogenic species. These organisms, however, are by no means limited to the mammals, but on the contrary, they may be found in almost all forms of life down to the insects.

Some of the earliest observations made upon trypanosomes were on those of the frog. At the present time a number of species are known to occur in these animals. They are nearly all characterized by their great bulky size, although long and slender as well as short forms are known. Williams and Lewis obtained a successful initial culture of the *Tr. rotatorium*, while more recently Bouet has given a detailed study of the cultural forms. Not only batrachians, such as frogs, but also reptiles, as turtles and snakes, have been shown to harbor trypanosomes in their blood.

Of particular interest perhaps are the flagellates present in the blood of fish either from fresh or from salt water. Of these two genera have been recognized,—*Trypanosoma* and *Trypanoplasma*. The latter genus was created by Laveran and Mesnil and includes forms which differ from the true trypanosomes in having a posterior as well as an anterior free whip.

The trypanoplasmata have been studied, particularly by Laveran and Mesnil, Brumpt, and by Keysselitz. The latter has observed a double infection with the two genera in fourteen species of fish. Although a considerable number of species of fish trypanosomes and trypanoplasmata have been described, Keysselitz regards the latter in the fish studied by him as representing but one species, *Trypanoplasma Borreli*. The percent-

age of naturally infected fish cannot be readily given, but there is reason to believe that it is very large. As in the case of birds, the flagellates may be present in but very small numbers and hence escape detection.

The infection is undoubtedly spread among fish through the agency of blood-sucking parasites and more especially the leeches. The studies of Brumpt and of Keysselitz have shown that a large percentage of the leeches contain variable numbers of flagellates in their intestinal canal. Owing to the many difficulties attending such investigations it has not been possible as yet to prove definitely that the flagellates observed in the leech are derived from those in the blood of fish or that conversely the fish flagellates develop from those multiplying in the gut of the leech. Nevertheless, it has been assumed and it is quite generally accepted, that the leeches are the intermediate hosts of the parasites. Keysselitz, and especially Brumpt, has succeeded in infecting fish by placing on them infected leeches.

It is an interesting fact that flagellates are present in the gut of many insects, irrespective as to whether these feed on blood or otherwise. The *herpetomonas* of the house-fly is an example of infection of a non-biting insect. Many similar observations could be given to show that intestinal parasitism by flagellates is a common occurrence. We have shown that 15 per cent. of the wild mosquitoes may be infected with *herpetomonas* and *crithidia*, while Ross, Legér and others have shown similar parasites in the larval and pupal stages. These two organisms when grown on blood agar retain the same form as observed in the insect, thus demonstrating that they are cultures *in vivo* and, as such, that they are really multiplication forms of trypanosomes and not distinct genera.

It follows from the above that much caution must be used in drawing conclusions as to the relation of flagellates found in insects to the blood trypanosomes or to the intracellular parasites found in vertebrates. As has been pointed out, the trypanosomes of tsetse-flies are not to be regarded as multiplication forms of *Tr. Brucei* and *Tr. gambiense*, and the same conclusion holds for Schaudinn's views regarding *Tr. noctuæ* and

Spirochaeta Ziemanni, which two forms were considered as flagellate stages of the halteridium and leucocytozoon of the owl.

The foregoing summary of the trypanosomatic infections would be incomplete without a brief reference to the flagellates of birds. These were first studied by Danilewsky, who described a large and a small form of *Tr. avium* (1885). Since then these forms have been found by a number of other investigators, and for the details of this work the reader is referred to the monograph on "Bird Trypanosomes" by Novy and MacNeal.

It has been shown by us that flagellate infection of birds is exceedingly widespread and that it can be recognized best by the cultivation method. A number of species have been shown to exist in our common birds. These can be readily cultivated and the characteristics presented by the cultures readily permit the differentiation of species. Since then Thiroux has been able to grow the *Tr. paddæ* and Cerqueira has been equally successful with the trypanosome of the *Nicticorax* of Brazil. The most important result of these studies on bird trypanosomes has been the demonstration that these flagellates are in no wise related to the intracellular parasites.

AUTOLYSIS*

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TISSUE DISINTEGRATION.

THE most constant property of living matter is disintegration. This precedes every other manifestation of life in the simplest and in the most complex animated organism. The development of a fertilized egg begins with disintegration of its original structure. When the egg is placed under conditions which make the initial disintegration impossible, life remains suspended. Disintegration also is the most lasting property of living matter, for when all other functions are extinct this property is still in evidence, and, if conditions are favorable, the disintegration proceeds until all evidence of organization or of structure disappears, giving place to a mixture of organic and inorganic substances.

Disintegration of organic matter present in the cells and tissues is also the primary source of that form of vital energy which controls most functions of the living organism. Contraction of muscle, secretion of glands, peristaltic movements of the gastro-intestinal tract, growth and reproduction of an organism, are possible only so long as the breaking-down process continues. This has been realized by physiologists at all times. However, the mechanism and the exact nature of the chemical reactions associated with animal functions have never been very clear, and even to-day the question remains a topic of considerable controversy.

Lavoisier was the first to emphasize strongly the similarity between the chemical reactions in the living organism and those in the process of combustion. Ever since that time physiologists have adopted the term combustion in order to signify

* Lecture delivered November 18, 1905.

the chemical reactions in the organism which result in the production of animal energy. Physiologists speak of burning or non-burning in the human or in the animal body of proteid, fat or sugar, of burning of the body tissues and of body cells. Indeed, the ultimate products of the reactions in the body are very similar to those from the burning of carbonaceous material. Carbon dioxid, water and heat are formed in both instances. However, there has always existed an utter lack of information regarding the agents causing the powerful oxidation of organic material in the animal body. The simplest way out of the difficulty seemed to ascribe the power of combustion to a peculiar property of the living cell.

In recent years there has accumulated a great number of observations tending to show that various functions previously regarded as the result of life, as the result of cell assimilation and disintegration by the animal tissues, are actually occasioned by substances which can be isolated from the living cell. An instance illustrative of this statement is found in the work on alcoholic fermentation. The formation of alcohol from grape sugar by the yeast cell was regarded as a chemical reaction brought about by the activity of the cell. Alcohol thus was considered a catabolic product of cell metabolism. Mme. Manassein, and, more convincingly, Buchner, have demonstrated that one substance from the cell can be obtained which is capable of accomplishing the alcoholic fermentation of sugar in the same manner as the living cell. This startling discovery marks a radical change in our conception of the process of life. For centuries it was thought that the activity of a complex organism was needed to manufacture spirits out of grape sugar, and the foregoing work demonstrated that the cell may be crushed, its life may be extinct, and one substance soluble in water may be extracted which possesses the power to accomplish the work of the entire cell. True, the substance is of a very subtle nature and needs to be handled with great care. It does not resist the action of heat and other strong chemical and physical agents, but while intact it is capable of inducing chemical reactions into which it apparently

does not enter itself, and thus is capable of performing work out of proportion to its own mass. Substances endowed with this power are designated enzymes or ferments. The work of Buchner caused a very intense interest to be directed toward the older and unsystematic work on the enzymotic processes in the cells of the simple and complex organisms.

As already stated, the source of all vital and animal energy lies in tissue disintegration, and the prevailing conception has been that the disintegration was brought about by the power of the living cell to burn its own components. It is the great merit of Salkowski to have shown that a cell or tissue in which all visible signs of life have disappeared still retains the power of self-dissolution, of self-disintegration, of autolysis. True, the phenomenon had not escaped the observation of earlier workers, and in 1871 Hoppe-Seyler wrote: ¹ "All organs suffering death within the organism, in the absence of oxygen, undergo softening and dissolution in a manner resembling that of putrefaction. In the course of that process, albuminous matter gives rise to leucin and tyrosin, fat to free acids and soaps. This maceration, identical with the pathologic conception of softening, is accomplished without giving rise to ill odor, and is a process similar to the one resulting from the action of water, acids and digestive enzymes." In 1874 the French chemist, Schutzenberger ² observed similar changes in yeast which had been allowed to remain for from 12 to 15 hours in water suspension at a temperature of from 35° to 40° C.

However, before going into the details of chemical analyses, attention may be called to the structural, morphologic changes which cells and tissues undergo when they are placed in conditions which do not permit continuation of life. It is well known that animal tissues and organs are readily invaded by micro-organisms, causing putrefaction. The application of

¹ Tübinger Med. Chem. Untersuchungen, 1871, p. 499.

² Compt. rend., vol. lxxviii, and Bull. de Soc. Chimique, vol. xxi, p. 204.

the recent methods of aseptic surgery allows the removal of organs from the animal body and the preserving of them free from all contamination with micro-organisms. Hauser,³ as well as Rindfleisch and Meissner previous to him, succeeded in preserving tissues for months and years free from infection with any bacteria. In organs kept in an absolutely sterile condition Hauser observed general softening, and microscopically he noted the destruction of the most typical structural part of the cell, the nuclear material, and decay of the mass of the cell, made apparent by the development of changes which are designated by pathologists as fatty degeneration.

Thus the term softening is not merely a figure of speech, but applies to an actual occurrence. This is made evident particularly through the work of Schutzenberger and the workers who followed him. Normal fresh organs on extraction with boiling water give off only a small fraction of their constituents, while those that have undergone the process of softening allow a very considerable part of their substance to pass into the boiling water. Thus, fresh yeast on boiling with hot water leaves a residue consisting of from 20 to 21 per cent. of its original weight, while the residue of yeast kept in water for from 12 to 15 hours does not exceed 13 per cent. However, in the experiment of Hauser, although the tissues were placed in conditions unfavorable for continuation of life, death set in slowly, and the possibility is not excluded that the softening was accomplished by the vital force not yet completely extinct. In the experiments of Schutzenberger also this possibility was not excluded; besides, yeast always contains bacteria and it is difficult to separate the part of the changes wrought by the action of micro-organisms from that induced by the surviving yeast cell.

Salkowski was the first to preserve the material employed in his experiments under conditions which checked all functions but that of dissolution, bacterial growth being impossible. This was achieved by the use of chloroform water instead of the

³ Arch. f. exper. Path. u. Pharm., vol. xx, p. 162, 1886.

pure. Salkowski repeated the experiments of Schutzenberger on yeast and arrived at the same conclusions as the first observer. He extended the work to animal tissues, using the liver and muscle. The results are best seen in the following table:

From 1000 grams of liver were extracted by hot water	Autolyzed organ	Control	Difference
Organic substance	45.97 gms.	33.73 gms.	12.24 gms.
Ash	7.95 gms.	7.21 gms.	0.74 gms.
Phosphoric acid	1.957 gms.	1.359 gms.	0.598 gms.
Nitrogen in form of nitrogenous substances	6.239 gms.	3.152 gms.	3.087 gms.

In the main experiment the finely-divided organ was mixed with three times its weight of chloroform water and allowed to stand. At given intervals analyses were made. In the control experiment the organ was heated and then further treated in the same manner as in the principal experiment. The table clearly shows that, on standing, substances soluble in hot water have developed in the organ. Very similar changes occur in tissues subjected to the influence of digestive enzymes, either in the digestive tract or outside of the body. Because of this analogy Salkowski introduced the term "self-digestion" in order to designate the process occurring in tissues allowed to stand under antiseptic conditions. For reasons which will be made clear in the course of the discussion, the process later was named by Hofmeister "autolysis."

Thus the researches of Salkowski have established the fact that tissues, placed in conditions which do not allow contamination with living matter, undergo changes resembling those occurring during the course of digestion; but they offered no information regarding the rôle played by the process in the economy of the organism, in those transformations of matter which create and maintain life. It was still undecided whether or not the capacity of self-digestion was a universal property of all tissues. The probability was not excluded that the autolysis of an organ was brought about by the action of enzymes absorbed from the gastro-intestinal tract and transported to the various organs. The researches following those of Salkowski

endeavored to give an answer to these questions. The solution of the first problem was comparatively an easy matter. It was necessary only to repeat his experiments on various other organs. This was accomplished most successfully by the efforts of Hedin and Rowland.⁴ It may be noted here that the last two investigators employed in their experiments not the entire tissue nor the tissue extracts, but the plasma of the organs. In this manner they made certain that no cellular elements were playing any part in their experiments, and that the reactions were caused by a soluble substance present in the plasma. Previously Schwiening,⁵ a pupil of Salkowski, had established the same fact by employing filtered tissue extracts. The work was further extended by Martin Jacoby⁶ and by Stookey and myself.⁷ As a result of all the work it may be regarded as established that the power of self-digestion is shared equally by all organs. The solution of the second problem, namely, of the origin of the autolysing power, required more ingenuity and perhaps more work. Attempts were made to obtain the desired information in various ways. If the digesting power present in the organs be due to a substance derived from the pancreas, the autolysis of organs must be influenced by the same factors and in the same manner as pancreatic digestion; further, if that assumption be correct, one would expect to find among the products of autolysis those substances which arise on tryptic digestion.

The chemical composition of animal organs is very complex, but the pancreatic gland is capable of disintegrating all the principal tissue constituents, although it resorts to a different mechanism, perhaps to a different substance, for the digestion of the individual substances. The principal components of tissues are albuminous material, carbohydrates and fats. In the course of self-digestion all these components are disintegrated, and it is a matter of convenience to discuss separately

⁴ Zeitsch. f. phys. Chem., vol. xxxii, 1902.

⁵ Virchow's Archiv., cxxxvi, 1894.

⁶ Zeitsch. f. phys. Chem., vol. xxx, 1900.

⁷ Jour. Med. Research, vol. x, 1903.

the change which each of the components undergoes in the course of autolysis. Of all enzymotic processes, that resulting from the breaking up of the proteid molecule has been studied in the greatest detail. For this reason the study of the proteolytic action of organs was employed for the investigations into the origin of the autolytic power of tissues.

Two proteolytic enzymes of distinct individuality have always been known—pepsin, elaborated by the glandular apparatus of the stomach, and trypsin, formed in the pancreatic gland. The principal point of distinction between the two substances is that one requires for its action the presence of acid, while the other is most active in the presence of alkali. Further, it has generally been accepted that pepsin is incapable of producing the same degree of cleavage as trypsin. The formation of crystalline products of amino-acids has been noted only on tryptic digestion. Most typical for the cleavage by the ferment of the pancreatic gland is considered the appearance of a substance giving a peculiar color test with bromin, named tryptophan. In the course of digestion by either of the two enzymes, albumoses and peptones are formed.

Biondi, a student of Salkowski, has noted that the proteolytic action of the liver is facilitated by the presence of acids. This difference in intensity of digestion under the two different conditions is made very conspicuous by the following table:

Out of 1000 grams of liver passed into solution	Experiment 1 with 0.28% HCl	Experiment 2 without HCl
Organic substances	100.10 gms.	59.0 gms.
Ash	26.90 gms.	11.12 gms.
N. in nitrogenous substances...	11.76 gms.	7. gms.
Albumose	Trace.	Trace.
Pepton	None.	None.

The conditions influencing the intensity of autolysis were studied in greater detail by Hedin and Rowland,⁴ whose investigations were made on tissue plasma obtained by Buchner's method. It was established by these writers that the self-digestion of the majority of organs is facilitated by the presence

of 0.25 per cent. of acetic acid and is depressed by the presence of alkalies, by calcium carbonate and magnesium oxid. The only deviation from this, according to Hedin and Rowland, is in muscle tissue, where the intensity of digestion is not affected by the presence of alkali or acid. On the other hand, cardiac muscle is subject to the general rule of autolysis. The autolysis of nerve tissue, and of the testes also, is facilitated by the presence of acid, as was demonstrated by Stookey and myself.

These observations are important, for the reason that they make very improbable the supposition that self-digestion of tissues is caused by trypsin deposited in the organs by the blood supply. On the other hand, Salkowski, in his early work on autolysis, has noted the appearance of leucin and tyrosin, and in this respect the proteolytic action of animal tissues resembles tryptic digestion. Contradictory to this seemed the observations of Biondi.⁸ This author could not detect tryptophan in the experiments in which the absence of bacterial growth was made certain. Another peculiarity of the autolytic cleavage noted by Biondi is the comparatively insignificant formation of albumose and of peptone. Jacoby also, in his very exhaustive study on autolysis, invites special attention to the foregoing difference between tryptic and autolytic digestion. On the other hand, Jacoby demonstrated tryptophan among the products of self-digestion of tissues. Thus the chemical process of autolysis bears some resemblance to either form of digestion, peptic and tryptic, and yet is different from each of them. This alone makes it very probable that animal tissues do not borrow their power of disintegration from either gastric or pancreatic gland, and that self-digestion is one of the general properties of living or, rather, surviving organs.

Additional evidence in support of these assumptions was brought forward by Matthes.⁹ It is well known that urine of normal individuals contains a proteolytic enzyme resembling

⁸ Virchow's Archiv., vol. cxliv, 1906.

⁹ Archiv. f. exp. Path. u. Pharm., vol. li, 1904.

pepsin. Matthes demonstrated that after the removal of the stomach of dogs the enzyme ceases to be eliminated by the urine. It was natural on the basis of this experiment to view the stomach as the source of the urinary pepsin. The same method of investigation was applied by Matthes to the study of the origin of the self-digesting power of organs and tissues. Dogs were deprived of their pancreas and allowed to recover from the operation. The organs were then examined for their proteolytic power. No difference could be detected between the organs of the normal and those of the operated animal. Thus, all evidence seemed unanimously to support the view that self-digestion is a constant property of surviving tissue.

However, for the interpretation of the rôle of this function in the economy of the living organism, it still remained to be established whether or not the process of self-disintegration takes place also in life. Jacoby⁶ was the first to give experimental trial of the question. For this purpose he performed on dogs the following operations: The hepatic artery and the portal vein were ligated and, after several hours, the liver was extirpated and analyzed for amino-acids. Leucin and tyrosin were found to be present. Further, he obtained the same results on ligating a part of the liver. These substances were also obtained by Jacoby from organs extirpated aseptically and kept under conditions in which contamination was impossible. It may be remarked that all these methods are open to some objections. More convincing seems to me the analysis of the developing organism. It has been known for some time, through the work of Schulze and his pupils, that in the course of germination and growth of plants, substances appear which arise also on proteolytic digestion of the seeds. I have made a similar observation on the developing egg of fish and of fowl.¹⁰ In the course of development of the egg one can notice the breakdown of the albuminous matter and the appearance of products of the nature of nitrogenous acids.

¹⁰ Zeitsch. f. phys. Chem., vol. xxxv, 1902.

So, at the present time, there is sufficient evidence for the assumption that disintegration or self-digestion is a constant occurrence in living as well as in surviving tissues. However, there is still a lack of information regarding the rôle of this function in the mechanism of life. In the animal tissue, organ or cell, one has to distinguish two different parts, one representing the organized mechanism controlling its function, the other consisting of various organic substances stored up or deposited in the organs, as a supply of fuel material. Blood plasma and lymph, which envelop every part of the organ, are not integral parts of its tissue. They only furnish the material which the organ may or may not use. White of an egg and the greatest part of its yolk are only building material for the developing organism.

In physiology there are two views regarding the production of animal energy. One is that a substance can not be utilized by a living cell unless it has been assimilated and transferred into organized cell substance. Liebig was the author of this theory and Pflüger most vigorously defended it. On the other hand, Carl Voit claimed that in higher organisms the principal supply of fuel material is furnished to the organs by the blood. The albuminous matter carried to the organs was named by Voit "circulating proteid." Opinions on the subject are still divided and it is possible that in a way both views are correct.

Since there was some foundation for the view that the process of autolysis is the one which controls tissue disintegration, it seemed important to make clear whether or not the mechanism is capable of breaking down albuminous matter derived from other sources than that of its own body substance. The first observation in this direction was made by Theobald Smith, who noted that fresh tissues removed from the organism under aseptic conditions were capable of digesting gelatin. On the other hand, Martin Jacoby¹¹ noted that during the process of liver autolysis, of the proteids only the globulins suffered a

¹¹ Zeitsch. f. phys. Chem., vol. xxx, 1900, also vol. xxxiii, 1903.

disintegration; and in a later work he observed that the self-digesting liver was completely incapable of digesting lung tissue. Thus, on the basis of this work, one would be led to the view that the process of autolysis is incapable of causing the digestion of circulating proteid, and that the two processes are totally independent of each other. However, Hedin ⁴ has shown that the spleen possesses the power to digest not only its own proteid material, but also the proteids of the blood. Thus the question still remains an open one.

PRODUCTS OF TISSUE DISINTEGRATION.

The work thus far reviewed possessed primarily theoretical interest only. It aimed to elucidate the mechanism controlling the disintegration of tissue components in the living and in the surviving organs. Nevertheless a detailed knowledge of the products of tissue autolysis is of importance from the standpoint of practical medicine. In the human organism, as well as in that of many animals, all substances which are consumed as food and nourishment, no matter how greatly they differ in their chemical composition, are finally broken down into a few very simple bodies, which are rejected by the organism through the kidneys, bile and other excretory mechanisms. Urea and carbonic acid are the two substances into which nearly all food-stuff is transformed. In a complex organism the metamorphosis is a gradual process. Before a nitrogenous substance is transformed into urea it undergoes numerous degradations. Before sugar is oxidized to carbonic acid it suffers numerous changes. Further, it is not improbable that in a very complex organism individual organs are concerned only in one definite phase of the transformation, leaving the other organs to continue and to complete the work. In his recent address on the subject, Professor v. Noorden ¹² pointed out that the information regarding the nature of intermediate products of metabolism, as well as the seat of their formation, is lacking. Attention of investigators has turned to the study of the products of autoly-

¹² This volume, page 18.

sis of various organs in the hope of filling in the gap in our knowledge of the mechanism of nutrition and of self-preservation of the organism.

However, the study of the substances arising in the course of autolysis was preceded by very active work on the normal composition of tissues and tissue components. Indeed, it was to be expected that within the body, tissue constituents would break down into their component parts. Recent years are marked by astonishing progress in the knowledge of the chemical nature of tissues. It was owing to this progress that the study of autolysis was made a comparatively easy matter. As already stated, the principal tissue components are albuminous matter, sugars and fat. The changes which each one of these components undergoes in the course of self-digestion have been the subject of special investigation.

Under the term proteid is generally understood the substance which represents the most important and most characteristic part of living matter. It is colloidal in nature and is composed of various nitrogenous acids. On heating proteid with strong acids or alkalies, the original substance disappears, giving rise to the nitrogenous acids. Of those already known are the following:

Glycocoll.	Lysin.
Alanin.	Arginin.
Aminovalerianic acid.	Histidin.
Leucin.	Prolin.
Glutamic acid.	Tryptophan.
Phenylalanin.	Cystein.
Tyrosin.	

Of the proteids, one group attracts special attention. Its members are present in greatest quantity in the nuclei of all cells, and it has been assumed that the function of the nucleus is closely associated with the presence of these substances. They are named nucleins, nucleoproteids, nucleoalbumins, etc. They are more complex than ordinary proteids, containing in their molecule, besides the usual constituents, a body termed nucleic acid. This acid is composed of substances to which a considerable rôle in the pathogenesis of disease has been attributed.

Its components are as follows: Phosphoric acid, carbohydrate, thymin, uracil, cytosin, adenin, guanin, hypoxanthin.

Normally, components of simple and complex proteids occur as such in tissues in very insignificant quantities. But it is found that in the course of self-digestion an organ may underge such deep changes that nothing remains of its original structure, in its place the following substances appearing:

	Pancreas	Liver	Spleen	Kidney	Testes
Glycocoll	—	—	—	—	—
Alanin	+	+	+	+	+
Aminobutyric acid	+	+	+	+	+
Aminovalerianic acid	?	?	+	?	+
Leucin	+	+	+	+	+
Glutamic acid	+	+	+	+	+
Aspartic acid	+	+	+	+	+
Pyrrolidin carbonic acid	?	?	+	+	+
Tyrosin	+	+	+	+	+
Phenylalanin	+	+	+	+	+

A glance at the table shows clearly that the action of the autolytic process in organs is as powerful as that of strong acids combined with high temperature. Nearly all the products which are obtained on prolonged boiling of proteids with strong mineral acids arise also in the course of autolysis. However, there are noted some differences in the two processes. If it be allowed to name substances appearing on cleavage with mineral acid as primary cleavage products, the distinction may be made that on autolysis the primary products undergo further transformation. It is a matter of convenience to discuss the points of difference according to the three principal groups of substances in which they occur, namely: 1. The nitrogenous acids containing only one nitrogen in their molecule, monoaminoacids. 2. Acids with more than one nitrogen in the molecule (The substances of this group arising from proteid cleavage were named by Kossel hexon bases. They generally possess basic properties). 3. Substances resulting from the nuclear degradation, nuclein derivatives or nuclein bases. The most

appropriate method for investigation was: first, to study the products obtainable on boiling organs with strong acids; second, to study those arising on autolysis of the same organs and, finally, to analyze the substances appearing on boiling with strong acids of organs previously subjected to self-digestion.

On acid cleavage all the amino-acids are obtained which are known to appear on the breaking down of proteid material. Among the end-products of self-digestion of the pancreas, Emerson¹³ discovered oxyphenylethylamin, which is not known to be present in the proteid molecule, and which may be regarded as a secondary product derived from tyrosin. Further, on autolysis of various organs the formation of glycocoll was not observed, and prolin could be demonstrated only in a few experiments. It should be remarked that the present methods of analysis of amino-acids are not fully satisfactory, and too much weight should not be attached to the results thus far obtained. However, the results of the analysis of the amino-acids obtained from the fresh and from the self-digested glands seem to indicate that in the course of the latter process some destruction of the substances takes place. This may be seen from a table showing the results of experiments not yet published, although completed:

	Fresh Spleen 5 pounds	Autolyzed Spleen 5 pounds
Glycocoll	0.700	0.700
Alanin	8.6	1.7
Aminobutyric and aminovalerianic acids	5.25	5.00
Leucin	14.75	12.0
Aspartic acid	2.24	0.8
Glutamic acid	3.12	1.25
Phenylalanin	1.15	1.33
Prolin	Present.	Present (inactive).

The knowledge of the further phases of amino-acid metamorphosis is rather meager. Stolte¹⁴ has shown that amino-acids exposed to the action of tissue extracts give rise to ammonia, and Magnus-Levy¹⁵ has demonstrated the formation of fatty

¹³ Hofmeister's Beiträge, vol. i, 1901.

¹⁴ Hofmeister's Beiträge, vol. v, 1904.

¹⁵ Hofmeister's Beiträge, vol. v, 1904.

acids in the course of autolysis. Diamino-acids and other basic substances of the proteid molecule suffer a similar disintegration. Thus, on prolonged autolysis of the pancreatic gland or of the gastric mucosa, the formation of diamins from diamino-acids, a process analogous to the transformation of tyrosin into oxyphenylethylamin, was observed by Lawrow,¹⁶ Langstein,¹⁶ and by myself.¹⁷ It has also been noted that a very considerable part of the diamino-acids suffers a more complete disintegration. Thus five pounds of fresh spleen yields on hydrolysis 3.2 gm. of arginin and 2 gm. of lysin and the same quantity of digested glands only 1.5 gm. of arginin and 1.2 gm. of lysin. The mechanism controlling this degradation was explained by the brilliant discovery of Kossel and Dakin.¹⁸ These authors have demonstrated in various organs the presence of a special enzyme whose function it is to decompose arginin into urea and diaminovalerianic acid. The same enzyme was found by Shiga in the yeast cell.

Before concluding the review of the products resulting from proteid cleavage in the process of autolysis mention has to be made of the formation of plasteins. From the foregoing discussion it is apparent that great activity has been displayed in the study of degradation of the proteid molecule within the body and in the test tube, and an attempt has been made to explain the bearing the work had on our understanding of various physiologic functions. However, there was one great problem which inspired many workers in their labors and which remains unsolved by them. This was to discover a process by which proteid, the chief tissue component, could be constructed out of its simple components, and also to discover the mechanism which the organism employs to build up tissue proteid out of those fragments which are formed in the digestive tract. It has been stated before that the first phase in

¹⁶ Zeitsch. f. phys. Chem., vol. xxxiii, 1901.

¹⁶ Ibid., vol. ii, 1902.

¹⁷ Amer. Jour. of Physiol., vol. xii, 1904.

¹⁸ Zeitsch. f. phys. Chem., vols. xli and xlii, 1904.

proteid digestion consists in converting native proteid, which is insoluble in boiling water, into products which are soluble both in hot and cold water. These products are termed albumose and peptone. Danilewsky and his pupil, Okunew,¹⁹ were the first to make the observation that when the soluble substances are exposed to the action of rennet ferment a substance arises which is insoluble in water. This substance was termed plastein. Kurajeff, another student of Danilewsky, has shown that the plasteins are formed by the action of autolytic enzymes also. It has been shown in recent years that enzymes possess a double function. They break up complex into more simple substances and again rebuild the original substances from the fragments. Hill made this observation on the enzymes digesting starch, and Kastle and Loevenhart²⁰ on that splitting fat. The first ferment is capable of converting sugar into starch and again starch into sugar; the second possesses the power of converting fat into fatty acids, and the acids into fat.

Attempts were made to ascribe a similar faculty to enzymes digesting proteids, and the plasteins were regarded by many investigators as reconstructed native proteid. The most emphatic supporter of this theory was Herzog.²¹ He based his assumption on a very ingenious experiment. The viscosity of a dilute solution of native proteid decreases in the course of digestion. On the other hand, the viscosity of a fairly concentrated solution of albumose exposed to the action of digestive enzymes increases. This, according to Herzog, is to be explained by the reconstruction of proteid. However, if the digestive action of the enzyme is disturbed by the presence of an antiferment, the reconstruction fails. Very recently Kurajeff and his pupil, Grossman,²² exposed digested plasteins to the action of autolytic enzymes and noted the formation of coagulable proteid, and they seem inclined to believe that this indicates a reversible

¹⁹ Maly's Jahresb., vol. xxv.

²⁰ Amer. Chem. Jour., vol. xxiv, 1901.

²¹ Zeitsch. f. phys. Chem., vol. xxxix, 1903.

²² Hofmeister's Beiträge, vol. vi, 1905.

action of the autolytic enzymes. Unfortunately the experiments of various writers on the subject contain many contradictions. While the primary albumoses are considered the mother substance of plasteins by some writers, Kurajeff noted the formation of plastein only from the secondary ones. Further, Bayer,²³ in Hofmeister's laboratory, found that plastein is formed from crystalline cleavage products.

In our own experiments, Stookey and I failed to find any evidence of reconstruction of the lower albumoses into coagulable proteid. By the action of rennet ferment on albumose it was possible to convert the solution into a solid jelly, which was very difficult to fractionate for purely mechanical reasons. If, for the experiment, a solution of albumose of moderate concentration was employed, it turned into a very thick syrup. In the original solution and the one treated with rennet the various albumoses were estimated. The time of treatment with rennet varied from 12 to 48 hours. In no instance was there observed any diminution in the quantity of the lower albumose or of the peptone. Thus there was no evidence of reversion of peptone into coagulable proteid, and it is extremely improbable that enzymotic synthesis of proteid can be made possible before the substances at present known as proteolytic enzymes are divided into their components. Cleavage of proteids by trypsin, pepsin or other proteolytic enzymes is a very complex process. Reversibility of action thus far is known to be a property of enzymes affecting a hydrolytic cleavage. In the course of proteid disintegration in the tissues and in the digestive tract, the primary products so rapidly suffer further metamorphosis that reversion on a large scale is scarcely imaginable. There must be other synthetical processes to which the organism resorts for the purpose of tissue construction.

It has already been stated that special attention has been directed to the study of the products of autolysis of nuclear material. Through the work of Fischer, it was established that the components of nucleic acid were closely related one to

²³ Hofmeister's *Beiträge*, vol. iv, 1904.

another and all of them, in turn, related to uric acid. In addition, the pathogenesis of many diseased conditions, and particularly that of gout, is closely associated with uric acid. Normally, fresh organs contain the components of nucleic acid in a free state only in a very insignificant quantity. Schutzenberger, Salomon and Lehmann (the last working under Kossel) observed that yeast suspended in water and allowed to stand at body temperature gave rise to free nuclein bases, the fresh cells not containing the substances in a free state. Salkowski and his pupils, Schwiening and Biondi, made it certain that the appearance of the substances was due to an autolytic process. More recent investigations have made it clear that through autolytic action purin bases, as soon as they become detached from the complex molecule of nuclein, suffer complete decomposition. Statements to that effect were made by earlier observers, more recently by Kutscher. The observation of Dakin²⁴ is in harmony with this statement, although he could detect in autolyzed kidneys only one purin base, hypoxanthin. The correctness of the statements was demonstrated by my own investigations. Quantitative analysis of all purin bases present in the fresh and in autolyzed organs has made clear the destruction of nuclein bases in the course of self-digestion. Very recently also some information has been gained regarding the chemical process of their disintegration.

In fresh tissues adenin and guanin occur in large amounts, the other two purin bases in very small amounts. Jones²⁵ and I,¹⁷ independently of each other, have shown that by autolysis, these first two bases are changed into hypoxanthin and xanthin, respectively. The following shows the contents of purin bases in five pounds of fresh and autolyzed spleen :

	Fresh Spleen	Self-digested Spleen
Adenin	1.85 gm.	0.0 gm.
Guanin	1.10 gm.	0.0 gm.
Hypoxanthin	0.30 gm.	1.2 gm.
Xanthin	0.40 gm.	0.150 gm.

²⁴ Jour. of Physiol., vol. xxx, 1903.

²⁵ Zeitsch. f. phys. Chem., xli, 1904.

Schittenhelm arrived at similar conclusions and demonstrated further that under certain conditions the bases are transformed into uric acid. The other constituents of nucleic acid, the pyrimidin bases, undergo analogous changes.

The mechanism of this transformation was elucidated by the work of Jones.²⁵ This author accepts the presence in the tissues of two specific enzymes, one capable of acting on guanin and the other on adenin, and further of an oxidizing enzyme, the function of which it is to complete the transformation of nuclear material. The deductions of Schenck²⁶ are similar to those of Jones, and Schittenhelm²⁰ has corroborated in a general way the same conclusions. However, he does not support the assumption of the existence of more than one enzyme which can transform the amino-purins into the corresponding oxy-derivatives. According to Schittenhelm, the entire nuclear destruction is accomplished by three enzymes, one breaking up the nucleic acid into its components, the second splitting off the nitrogen from the nitrogenous constituents, and the third completing the oxidation of the purin derivative. It must be admitted that more detailed information concerning the intermediary products of nuclein metabolism is still wanting.

With this I wish to conclude the review of the products arising on autolysis of surviving organs. Reference should be made to the products of autolysis of sugar and fat, but thus far the investigations in that direction are few in number and the results obtained from them not very significant. This also concludes the review of autolytic action in normal organs. It remains to discuss these results in connection with the original problems which led to all these numerous investigations.

DISCUSSION OF RESULTS.

It has been stated already that the principal object of the work was: 1. To elucidate the nature and the mechanism of those chemical reactions which make the functions of the body possible. 2. To interpret the rôle of individual organs in the

²⁶ Zeitsch. f. phys. Chem., vol. xliii, 1904.

animal metabolism. 3. To study the intermediate products of metabolism, since there is a general agreement that by the accumulation in the organism of these substances many diseased conditions are occasioned.

The foregoing review leads one to the conclusion that the knowledge of intermediate metabolism has been furthered considerably. On the other hand, a comparative study of the products of disintegration of various organs fails to bring out marked differences among them, although, during life at least, some organs are known to be the seat of special chemical reactions. This leads one to the assumption that in the animal body the process of self-digestion does not control all chemical reactions occurring in organs, perhaps even not all the processes of disintegration. A most conspicuous instance illustrating this statement is found in the work in which Eck's fistula was employed. By this name is designated a fistula between the vena cava and the portal vein. The aim of the fistula is to exclude the liver from the portal circulation. The organism of the dog possesses a very intense power of burning uric acid; the acid is present in the urine of this animal only in traces, even after injection of two to three grams of the substance. Dr. Sweet and I have demonstrated that animals kept for weeks on a diet free from all precursors of uric acid excrete considerable quantities of it in the urine as soon as an Eck fistula is performed on them. The output is especially increased after the administration of the substance itself.

Evidently under these conditions the organism fails to disintegrate uric acid, although the process of self-digestion is not depressed in the tissues. Thus the mechanism of "burning" uric acid in the living organism is not known yet. Proteid combustion also in the normal living organism apparently is different from the proteid disintegration in the course of autolysis. The great mass of products in the process of self-digestion remain in the stage of nitrogenous acids. A small part of them lose their nitrogen and a still smaller part give rise to carbon dioxid. In the living organism the splitting off of nitrogen from proteid material is a very rapid process, and

the transformation of all carbonaceous material to carbon dioxide also occurs with much greater rapidity than it possibly could take place in the course of self-digestion. But it has been stated that disintegration by the process of autolysis does occur during life. This and the foregoing are not contradictory to each other. Every tissue consists of cells of different age, of different states of nutrition and of different resistance. Work on hemolysis has brought out most clearly that individual blood cells vary in their vulnerability. Cells in a state of defective nutrition succumb to the process of self-digestion. In the course of that process enzymes are liberated which are capable of digesting extraneous material also.

It is difficult to demonstrate the correctness of this view on a complex organism, but it is made very clear from observations on the yeast cell. It is the function of that organism to convert grape sugar into alcohol. So long as conditions for this function are favorable there is little evidence of the process of self-digestion in a colony of yeast cells. But as soon as conditions are so altered as to make the normal life and the alcoholic fermentation impossible, a very active proteid-splitting enzyme is developed by the yeast cell which causes digestion of the cell proteid and of other proteid material. In the living organism the two forms of metabolism undoubtedly coexist. One is the result of the function of the organs, the other of their disintegration. The supply of energy required for the maintenance of life is furnished possibly by the first process. It has already been stated that by means of autolysis proteid is converted principally into amino-acids. By this conversion proteid could not furnish the organism with its full calorific requirement. On the other hand, autolytic enzymes may act on cell proteid and on the surrounding proteid in a manner similar to that of the enzymes of digestive glands, namely, rendering them a more suitable material for rapid combustion.

It is marvelous that, notwithstanding the presence of destructive agents in all tissues, organs succeed in guarding their integrity. A most clever investigation of recent years throws light on the mechanism by which this is accomplished. The

integrity of the gastric wall, the function of which is to elaborate digestive enzymes, has been the cause of much speculation. Weinland²⁷ demonstrated that this was due to the presence of an antiferment in the digestive glands. In the blood also were found antitryptic substances by Hahn,²⁸ Landsteiner,²⁹ Glaessner³⁰ and Cathcart.³¹ The same property was noted in tissue extracts by Dr. Stookey and myself.⁷ Furthermore, we have demonstrated that tissue extracts exercise an action antagonistic to that of autolytic enzymes. However, in health the two tendencies are so regulated that the tissue disintegration is sufficient to permit the organs to perform their function, while excessive wear is avoided. But as soon as the normal nutrition of the organism is disturbed the autolytic power of tissues increases. The mere fasting of an animal suffices to occasion in the tissues an exaggerated tendency for self-destruction. This was demonstrated by the experiments of Lane-Claypore and Schryver. It has been known for years that in cases of starvation animal organs lose in weight and that the loss varies in different organs. It is not improbable that products formed by disintegration of some organs serve to support the integrity of other more important organs. More marked is the high destructive power of tissues in diseases of a grave nature. Thus, in diseases of the respiratory system and of the heart, an intense self-digesting tendency of the tissues was noted by Schlesinger. In infectious diseases a similar observation was made by Flexner.³² The work of this author preceded that of Schlesinger, and is very important for the reason that it furnished an interpretation for some old observations of pathologists.

Flexner demonstrated an unusually high rate of self-digestion in organs removed from individuals who succumbed to typhoid

²⁷ Zeitsch. f. Biol., vol. xlv, 1903.

²⁸ Münch. med. Wochft., 1903.

²⁹ Cent. f. Bacteriol., vol. xxvii, 1900.

³⁰ Hofmeister's Beiträge, vol. iv, 1903.

³¹ Jour. of Physiol., vol. xxxi, 1904.

³² Univ. of Penn. Bull., July, 1903.

fever and other infectious diseases. The observation on typhoid is of special interest, since the exaggerated autolysis in the course of the disease can not be ascribed to the action of the micro-organism, for it is known that the proteolytic power of that germ is very slight. That this high rate of self-digestion is not merely a post-mortem phenomenon may be concluded from the old clinical observation, that products of proteid digestion are eliminated by the kidneys in the course of infectious diseases. The occurrence of peptonuria in these pathologic forms is not infrequent. Thus, in the light of the new investigation, this symptom acquires a special significance. So long as the nutrition is in a sufficiently good condition to prevent wasting of the tissues of the body, peptone is not present in the urine.

However, the softening and the wasting of tissues is most striking when these are under the influence of protoplasmic poisons. For this reason the study of phosphorus poisoning has attracted great attention. Martin Jacoby⁶ first pointed out the high rate of autolysis of the organs removed from animals killed by phosphorus poisoning.

In this condition self-digestion takes place not only in surviving organs, but also during life. This may be concluded from the work of Abderhalden²¹ and his co-workers, who recently have demonstrated the presence of crystalline components of the proteid molecule in the urine of animals poisoned with phosphorus.

The importance attached to the study of phosphorus poisoning is largely due to the resemblance which the clinical symptoms of this condition bear to that of a spontaneous pathologic form known as yellow atrophy of the liver. While the morphologic changes in the liver in the two conditions are not absolutely identical, still they present many points of similarity. The most striking are the disintegration of cellular elements and the so-called fatty degeneration of the organ. Through the work of many investigators, and particularly through that of Wakeman and Waldvogel, it has become evident that the changes which the liver undergoes in these pathologic conditions are identical with those which the organ suffers in the course

of autolysis. Indeed, the fact that the liver of persons who have succumbed to yellow atrophy contains products of proteid digestion was demonstrated by Salkowski more than twenty years ago, and was recently corroborated by Alonzo Taylor.³³ However, in neither of the two forms are the changes limited to one organ. Jacoby has demonstrated that the blood in phosphorus poisoning presents a striking loss of coagulability. Still more striking is the power it possesses of liquefying coagulated blood. This peculiarity was interpreted as being due to the presence of a proteolytic enzyme in the blood. In the course of yellow atrophy, products of a proteid cleavage have been found in the blood by Frerichs. This has been corroborated by many investigators, and very recently Neuberg and Richter³⁴ have shown that leucin, tyrosin and lysin may be present in the blood in quantities which clearly show that their origin could not be limited to the liver alone.

Thus, in the foregoing forms all tissues are apparently affected in the same manner. Marked autolysis in them may be considered a symptom of decline in general health and nutrition. However, there are conditions in which self-digestion is located in one organ only; thus the atrophy of the thymus, the involution of a puerperal uterus, are accomplished by a process of autolysis. The softening of tumors is brought about by the same mechanism. This was made clear through the work of Petry,¹⁵ who demonstrated that freshly removed tumors contain products of proteid cleavage. The same author further demonstrated that the rate of self-digestion of the new growth is higher than that of a normal tissue. An attempt was also made to study the toxicity of the products resulting from this process. However, neither from a chemical nor a pathologic point of view could a difference between the end-products of autolysis of tumors and those of normal tissues be detected. Indeed, the intensity of self-digestion is high in all organs composed of cellular elements endowed with rapid growth.

³³ Jour. of Med. Research, vol. viii, 1902.

³⁴ Deutsch. med. Wochft., No. 16, 1904.

The occurrence of local autolysis is not, as a rule, productive of a lowering in general health. On the contrary, it tends to restore normal conditions when these have been disturbed.

There are other conditions in which the process of autolysis is of aid to the organism in the efforts to maintain its integrity. It has been stated already that in the course of infectious diseases tissues possess a high power of autolysis. Investigations of Blum,¹⁴ Conradi³⁵ and Levaditi³⁶ have shown that autolysis may be one of the means to which the organism resorts in order to elaborate protective substances. Substances of two distinct groups are formed in the organism as the result of infection. Those of one group aim to destroy the micro-organism and are designated bactericidal; the purpose of the other is to neutralize the toxin elaborated by the micro-organism—these are commonly named antitoxin. Normal tissues in course of autolysis may give rise to substances of either group.

Blum has shown that the products of autolysis of lymph glands possess the power to neutralize tetanus and diphtheria toxins and cobra venom. The mechanism of this action is not based merely on the physical properties of the autolyzed gland, for it is possible to save animals from death by injecting the products of autolysis subsequent to the injection of toxin.

Further, Conradi has tested the bactericidal power of the products of self-digestion of various organs. This author noted that the last, added to a suspension of bacteria in broth, prevented their growth. The intensity of bactericidal power varied in different organs, as presented in the following table:

Muscle	Strong
Lymphatic gland	Strong
Liver	Strong
Spleen	Strong
Thymus	Marked
Suprarenal	Marked
Bone marrow	Slight
Pancreas	None
Thyroid	None

³⁵ Hofmeister's Beiträge, vol. i, 1902; vol. v, 1904.

³⁶ Ann. de l'Inst. Pasteur, vol. xvii, p. 186.

Glancing over the table, one is struck by the fact that organs rich in leucocytes are most efficient in elaborating protective substances against infection. And one is naturally led to the analysis of the rôle played by the white blood cells in the effort of the animal body to maintain its integrity. A review of all the information gained concerning the action of leucocytes on the tissues shows that this is similar to the action of the digestive tissue enzymes. A tissue invaded by the white blood cells is subject to the action not only of the local enzymes, but of those of the blood cells as well. Digestion caused by the last, strictly speaking, cannot be regarded as self-digestion. However, the two processes present so much similarity that they are classified by most writers under the same head of autolysis.

The formation of proteid-digestion products through the action of leucocytes was first noted by a Russian writer, Eichwald, in 1864. The observation has been corroborated by many scientists and clinicians. Peptone has been demonstrated in pus and in the urine in all conditions associated with abscess formation. But the work that has attracted most attention and stimulated most research is that of Friedrich Müller. This author has demonstrated that in croupous pneumonia the resolution of the exudate is accomplished by a process of autolysis. The products arising in the course of resolution are identical with those occurring in proteid digestion. The mechanism of this process has been interpreted by Flexner, who, studying the intensity of autolysis in different stages of croupous pneumonia, observed that the intensity was very high in the stage of gray hepatization and very low in the stage of red hepatization, and explains this by the abundance of leucocytes in the exudates in the first condition and their scarcity in the other. Flexner also noted that in unresolved pneumonia the autolytic power is very imperfect. In this condition, as is well known, the exudate is very poor in leucocytes.

Flexner is inclined to interpret the failure of absorption of the exudate in unresolved pneumonia as being due to the disproportion between the leucocytes and the other constituents. Indeed, the correctness of the assumption that the absorption of

an exudate is accomplished by the action of leucocytes was established by the work of Opie,³⁷ who has noted that a fresh exudate does not possess the same degree of autolytic power as an old one. He has also noted that the lack of self-digestion in the first is due in some degree to the presence in the serum of a substance checking the digesting action of the blood cell. The rôle of the leucocyte in the absorption of inflammatory exudates had been previously described by Ascoli and Mareschi³⁸ and by Umber,³⁹ but the details of the mechanism were not fully understood until the appearance of the work of Opie. The discovery of the substance capable of keeping in check the destructive work of the blood cell is of great value for the understanding of the process. And it is now established that in the white cell the organism finds its most active factor for repairing the damaged and inflamed tissue.

There still remains to be discussed one point in the mechanism of leucocytic action. Regarding other tissue enzymes it has been stated that the destruction of the cell always precedes their liberation. Many writers have surmised that this applies also to the white blood cell. And yet this has not been fully established. The observation that in leukemia autolysis is noted during life seems to contradict the foregoing assumption. However, it is possible that the dead cell exercises at least a greater digestive action than the living. Hedin and Opie have demonstrated that from the white cell two enzymes can be obtained. One resembles the enzyme elaborated by the pancreatic gland, and the other the autolytic enzymes. It is conceivable that the first is secreted during the life of the cell, the last liberated only after its death. It is possible also that, once set free, the autolytic enzyme increases the power of the other enzyme. An analogous action of the spleen on the pancreas has been assumed by many writers and was proven by

³⁷ Jour. of Exp. Medicine, vol. vii, 1905.

³⁸ Maly's Jahresb., vol. xxxii, 1902.

³⁹ Münch. med. Wochft., No. 49, 1902.

Stookey and myself. Thus waste and repair are controlled to a large extent by the same factors.

Autolysis, then, is the process by which dead tissue is not only prevented from becoming a burden to the organism, but, in addition, becomes converted into useful fuel material. By the aid of this process diseased tissues, pathologic formations and new growths are removed and replaced by normal healthy tissues. The organism resorts to this process also for elaboration of protective substances against bacteria and bacterial toxins. It still remains to be demonstrated whether or not in the course of autolysis enzymes are formed which are concerned in producing that energy which makes all animal functions possible.

A CRITICAL STUDY OF THE RESULTS OF SERUM THERAPY IN THE DISEASES OF MAN *

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TEN years have elapsed since diphtheria antitoxin was introduced for the treatment of diphtheria. Hopes were entertained that the proof of its value established a general principle by following which we would be enabled to develop curative sera for all infections. These hopes have not been fulfilled. Diphtheria remains the only disease due to a micro-organism in which serum therapy is of great and practically universally acknowledged value. In tetanus the immunizing value of the antitoxin is as certain, but its curative value is limited, and, in fact, is disputed by many. In bubonic plague, septicemia, pneumonia, dysentery, typhoid fever, tuberculosis and other diseases the curative value of sera is slight or wanting. Nor is this all, for we no longer think it is but a question of time when the technical difficulties will be overcome and curative sera for all infectious diseases become available. We rather fear that present knowledge indicates that effective sera for well-developed infections will never be obtained for most diseases. This does not mean that efforts should slacken, but it does caution us that any statements claiming that marked curative effects have been obtained from newly developed sera should be received with skepticism until at least properly controlled experiments have led to undoubted results. That advances are still possible is clearly indicated by the recent successful use of serum treatment in Graves' disease and hay fever.

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Before trying to estimate the results of serum treatment in the diseases in which it has been used, let us consider the general principles underlying it, so that we may more intelligently approach the subject.

A serum to be efficient for the treatment of any infectious disease should contain either antitoxins capable of neutralizing sufficient of the poisonous substances produced by the micro-organism to prevent serious poisoning of the infected person, or germicidal substances able, alone or in connection with the body cells and fluids of the infected person, to destroy the micro-organisms.

Cells produce many varieties of toxins, and for only a portion of these have antitoxins been obtained. One broad division of toxins is into soluble extracellular toxins and endotoxins. Only two micro-organisms seriously infecting man produce these extracellular toxins to an important extent, namely the diphtheria and tetanus bacilli. A peculiarity which seems to belong to all of these extracellular toxins is that when they come in contact with the cells of certain animals such as the horse they cause the production of specific antitoxins. These antitoxins being specific are able to combine only with the toxins that stimulated their formation. This union produces as definite a neutralization as when sulphuric acid combines with calcium. A further important fact is that great quantities of antitoxins can be developed. The stimulus of repeated injections of these toxins in horses is found to produce such an immense accumulation of antitoxins in the blood, that ten to twenty cubic centimeters may contain sufficient antitoxin to neutralize all the poison that can possibly be found in the severest case of human infection. So far as preventing the poisonous action of these toxins is concerned it is simply necessary to get a large enough quantity of the antitoxin into the blood before sufficient toxin has passed from it to the cells. The toxin that is firmly united to the cell protoplasm will have passed out of reach of antitoxin. It is the late detection of tetanus poisoning which alone prevents the treatment of tetanus from being as successful as that of diphtheria.

Unfortunately for the value of serum therapy most bacteria produce chiefly endotoxins, and the majority of these bacterial poisons are so constituted that they do not stimulate the formation of antitoxins. All bacteria contain a mixture of different endotoxins, some of which in each organism are capable of stimulating the development of substances which neutralize their poisonous effect while others are not. Even in the case of diphtheria bacilli these non-neutralizable endotoxins are present, as shown by the fact that if the bacilli are washed free of their extracellular toxin and killed by chloroform, they are still slightly poisonous. One-half gramme of the dead bacilli injected into a full-grown rabbit is sufficient to kill it. Practically none of this poisonous substance is capable of producing antitoxin. If in diphtheria sufficient of these endotoxins were absorbed no amount of antitoxic serum would save life. Fortunately, except perhaps in the case of severe general broncho-pneumonia, such absorption never occurs. The extracellular toxin produced by the bacilli in an infected case is probably a thousand times as much as the endotoxins, so that in cases in which antitoxin is omitted the soluble toxins produce death before the endotoxins cause appreciable symptoms. With the exception of the diphtheria and tetanus bacilli the poisons developed by the other bacteria-producing diseases in man, such as the colon, typhoid, tubercle, and glanders bacilli, and the pneumococci and streptococci, are mostly endotoxins. In infections with these bacteria we have a considerable portion of the lesions produced by poisons for which neutralizing substances have never been obtained. Unless we discover some way to produce antitoxins for these bacterial poisons our only hope is to develop an efficient bactericidal serum. Now, as we know, many of these bacteria do stimulate in the infected body the development of substances which when they come in contact with the bacteria unite with parts of their protoplasm and so render them liable to attack by serum ferments and leucocytes present in normal blood. The substances which sensitize the bacteria to the serum ferment or complement are in general classed as antibodies or immune bodies, while those

which render them susceptible to the phagocytes are called opsonins.

A serum which is bactericidal when fresh will soon lose the power to destroy bacteria unless there is added to it a supply of fresh normal serum. Even then its power is not fully restored. In the case of an antitoxic serum the elements which make for success or failure are comparatively simple. It is merely a question of giving it in sufficient amount and early enough to have it meet the toxin before the latter reaches the cells. With a bactericidal serum the factors are much more complex. The bactericidal serum must for its part have sufficient of the sensitizing substances to act upon the quantity of bacteria present in the infected body. This is practically impossible since it is very difficult to accumulate large quantities of immune bodies in the blood and also because certain of them, such as the opsonins, quickly alter and lose their protective properties. Even a serum sufficiently rich in these bodies may nevertheless utterly fail, for the serum-bathed bacteria, according to their kind, must meet in the diseased person's blood either sufficient complement or sufficient active phagocytes. Unfortunately even in normal persons the complement substance and the phagocytes are present in insufficient amount to attack such a number of bacteria as is present in a serious infection. To make the matter worse, the amount of complement and the activity of the phagocytes decrease as disease advances, so that when they are most in demand they are least present. We know as yet of no way to increase appreciably the amount of complement or the activity of the phagocytes except by improving the general condition of the body.

Further hindrances to the bactericidal effect of the serum are the changes which develop during the progress of disease in the infecting micro-organisms. These changes may render them immune to these germicidal substances. That portion of the protoplasm of the bacterial cell which has an affinity for the antibodies of the bactericidal serum may under certain conditions hardly develop at all. This has been most extensively studied in connection with the special type of antibodies known

as agglutinins. It has been noted that bacteria which agglutinate readily when taken from cultures cease to do so when they are grown for some weeks in agglutinating serum, whether in the test tube or in the animal body. These bacteria grow without developing the substance which unites with the agglutinin. This is shown by the fact that when these bacteria are placed in the solution of agglutinin, that substance is not absorbed. Experiments have shown that this same inhibition in growth is true, at least to some extent, for other antibodies. If bacteria growing in our bodies change so as to develop less of the substances by means of which a bactericidal serum acts upon them, then to a greater or less extent a previously active serum becomes ineffective. Such changes in bacteria are believed to account, in part at least, for the survival of bacteria in blood which is bactericidal for laboratory cultures and may also explain relapses, as well as chronic septicemias and chronic local infections. It is probable also that bacteria can adapt themselves to resist the destructive influence of the phagocytes as well as the protective substances in the serum. Tubercle bacilli and gonococci normally possess to some extent this characteristic and possibly partly for this reason they continue as persistent infections.

There is another unfavorable influence which serum therapy must encounter. It is that the serum used must be taken from a different species of animal. Ransom was the first to show that when a bactericidal or antitoxic serum obtained from an animal was injected into another species the passive immunity was of a much shorter duration than when injected into the same species. When repeated injections are given another difficulty is encountered. It was found that an animal which had been injected with the serum of another species would develop what might be called antiproteids for the serum proteids of the species whose serum was injected and that these antiproteids would render inactive all the antibodies of this species. Pfeiffer found that an anti-cholera serum prepared by immunizing a goat against the cholera spirillum was rendered entirely inactive by adding to it rabbit serum from animals

which had been injected either with anti-cholera goat serum or normal goat serum. Bordet studied these phenomena carefully and reached the following conclusions:

“ There exist in the serum of untreated normal animals substances which, though not identical with the specific immune bodies appearing after immunization, are yet chemically very closely allied to them. It is on account of the existence of these substances that normal sera have to a slight extent the properties that immunization renders so energetic and at the same time so specific.”

A striking example of the neutralizing effect of antisera on antitoxins is the following: If guinea pigs which at intervals of a week have received three injections of five cubic centimeters of horse serum are given an injection of antitoxic horse serum, it is found that after twenty-four hours the antitoxin is wholly destroyed. The blood of guinea pigs not previously treated with serum remains, after a similar injection, antitoxic for several weeks.

This teaches that the blood of persons who have received injections of horse serum within recent periods destroys the antitoxins and other antibodies introduced in later injections much more rapidly than does that of persons who have not been so treated.

This acquired characteristic of the blood developed by previous injections adds another difficulty to the serum treatment of diseases, where as in tuberculosis many repeated injections are required.

The bactericidal effect of serum in animal tests frequently does not equal that in test tube experiments, for the reason that the dilution of the antibodies is so great in the former case, unless we inject larger quantities of serum than are safe. The antitoxin and the bactericidal substances are diluted as many times as the bulk of the fluids in the person treated is greater than the amount injected. As we have seen, this dilution is still further increased through the gradual destruction and elimination of the curative substances. There is also steady loss in strength when the serum is kept.

The last point I wish to touch on is the frequent difficulty of knowing what micro-organism is invading the body. Even bacteria which we consider identical are often, from the serum standpoint, diverse; thus there are many varieties of pneumococci, each of which requires different sensitizing substances. This difficulty has been partly met by producing so-called polyvalent sera through injecting into the same animal many varieties of the organism.

This brief and incomplete review is sufficient perhaps to make clear that the processes brought into play in the struggle between the infected body and the invading cell are extremely complicated. It is almost impossible to accurately study these processes in the animal body and so the attempt has been made to substitute the test tube for the animal as much as possible.

Much information has been unquestionably thus obtained, but there are limitations in the method, for leucocytes and serum removed from the body are not the same as in the circulating blood nor are bacteria taken from growth on culture media identical with those developing in the fluids of the body.

With all the knowledge obtained from investigations we are still largely in the dark as to why in one case the body succumbs to disease and in another it sooner or later destroys the invading parasite.

THE EFFECTS, ON MAN, OF INJECTIONS OF HORSE SERUM.

“SERUM SICKNESS.”

It was early noted that injections of antitoxic or bactericidal sera were sometimes followed after some days by a peculiar train of symptoms, principally rashes, pains in the joints, and fever. One of the first to publish his observations was Lublinski (*Deutsche Med. Wochenschrift*, 1894). His patient was an eight-year-old girl who received three injections of 10 c.c. each of diphtheria antitoxic serum on the second and third day of the disease. On the fifth day of the disease there was reddening of the site of injection. Nine days after the last injection there developed high fever and painful swelling of the joints, accom-

panied by an extensive macular exanthema of the multiform type. This condition lasted about four days. The child made a good recovery.

The next number of the same journal contains four cases of this condition reported by Scholz, and from that time on a large number of authors describe serum rashes.

Even at that early date the view was expressed that the phenomena were due to serum constituents other than the anti-toxin, and this was subsequently confirmed experimentally by injecting normal persons with normal horse serum. Out of 22 normal persons so injected fever occurred eight times and an exanthema 12 times.

Hartung in 1896 (*Jahrb. für Kinderheilkunde*) collected statistics on the frequency of serum rashes and found that they occurred in from 8 to 11 per cent. of the cases. He thought that the frequency was dependent more on the individuality of the horse furnishing the serum than on the amount of the serum injected. Since then this subject has received much attention and is of sufficient importance to consider in detail.

In the following account free use has been made of the excellent study on serum sickness recently published by v. Pirquet and Schick (*Die Serumkrankheit*, Deuticke, Leipzig, 1905).

Aside from a little local irritation one usually notices no immediate changes even after large injections; and during the next few days the site of injection shows absolutely no reaction. There is nothing to denote that a foreign substance has entered the body—the patient's general condition is not disturbed. Then suddenly, in about 20 per cent. of the cases, usually between the eighth and the twelfth day, but sometimes as early as the second or as late as the twenty-first, symptoms of disturbance manifest themselves.

Now and then one sees *prodromal signs* which precede the general outbreak of the symptoms by a few days. The skin is sensitive to even slight irritation and shows indistinct reddening; this is especially the case at the site of injection and its vicinity, which is reddened and itchy. The most constant of

the prodromal symptoms is a slight swelling of the nearest lymph nodes.

As a rule the real outbreak shows itself in the development of an eruption (which is generally urticarial in character) appearing first usually at the site of injection and soon over the rest of the body. In most instances it appears simultaneously in symmetrical places. Some of the wheals are pale, some surrounded by a livid red zone. The latter is especially the case if the patient scratches a good deal. If the lesions are close together, one often sees areas in which the eruption is confluent. In that case the entire area seems to be edematous. When this occurs in the face it often gives rise to marked disfigurement. Owing to the intense itching the patients are restless and out of sorts.

All of the eruptions are fleeting, a particular wheal lasts only a few hours and the entire urticarial rash rarely longer than from two to three days. There is no regularity in the farther course of the eruption, such as is observed, for instance, in small-pox.

The rashes are extremely varied in character, the skin being almost inexhaustible in the production of peculiar pictures. Hartung divided the rashes into the following four groups:

- (1) Urticarial
- (2) Scarlatina-like rashes
 - (a) Diffuse erythematous
 - (b) Finely sprinkled true scarlatiniform
- (3) Morbilliform
- (4) Polymorphous, including exudative forms.

Almost every observer, however, will have encountered cases which would not admit of classification even under these many varieties.

In the meantime, showing that the rest of the organism is taking part in the reaction, the temperature has risen more or less. The intellect and the special senses, however, are unaffected; the pulse is frequent in conformity with the temperature, but is of good quality.

In addition, however, to the course as just pictured and to

which the name "serum rash" has been given, there are other symptoms. The lymph nodes draining the site of inoculation in the cases which react, gradually enlarge from the period of incubation. With the onset of the fever and of the rash, this swelling may in its further course affect also other lymph nodes. The swelling is painful and tender to touch. It subsides when the serum phenomena have passed the height of their course.

Especial attention is called to the state of the renal functions and to the development of slight *edemas*.

With the edema there are usually no *urinary changes* which could indicate disease of the kidneys. Only in a minority of cases does an excretion of albumin in the urine occur. The amount of albumin, however, always remains small.

Compared with the constancy of the above-named symptoms the others are much less frequent. Of these the *joint pains* have always been regarded as one of the most prominent symptoms. They occur in about 1 per cent. of the cases.

The *general condition* of the patient is usually not much affected, especially when the disease lasts but a short time; in fact, the patient's feelings are often in marked contrast to the high fever. There are cases, however, in which after injections of large quantities of serum the disease lasts from four to five weeks. Here we often see prostration and loss of weight. As soon as the disease ceases, however, convalescence begins, the lost weight is rapidly regained and the disease disappears without leaving even a trace behind. No after-effects are observed, and not one of the individual's symptoms can lead to permanent injury.

V. Pirquet and Schick have never seen a fatal ending which could be ascribed to the action of the serum. At the most they concede that the serum sickness developing in cases severely ill which are treated with serum may so lessen the resistance of the patient that he succumbs to the combined attack. My experience coincides with theirs, except for those rare cases where immediate disturbance occurs. As is stated in detail later, there have been several of these ending fatally in which death must be considered as having been caused by an injection of

serum. In these the fatal ending has occurred within a few minutes of the injection.

THE BLOOD CHANGES IN CASES DEVELOPING SERUM SICKNESS.

In healthy individuals injected with antitoxin Billings found the red blood corpuscles to show a slight reduction in number in about one-half the cases. The hemoglobin was correspondingly affected. The leucocytes were apparently unaffected by the injections. Practically identical with these findings were the results obtained by Field of our laboratory in some two dozen normal children injected either with ordinary or with refined antitoxin (Gibson's process).

In many cases developing serum symptoms, after large doses or moderate doses, v. Pirquet and Schick describe a marked leucopenia developing with the onset of these symptoms. The decrease is particularly at the expense of the polynuclear cells and resembles closely the leucopenia observed in measles, scarlatina, and vaccinia.

THERAPY AND PROPHYLAXIS.

The therapeutic treatment of serum sickness may be dismissed with the words "symptomatic treatment." Cold compresses to the swollen parts and the affected joints, prolonged warm foot-baths, local applications to relieve the itching, sponging for the fever, etc.

So far as the prophylactic treatment is concerned we must remember that the serum sickness is due to certain constituents of the serum, and that these have apparently nothing to do with the antitoxic or protective properties of the serum, for normal horse serum produces the same effects as does antitoxic serum. As soon as this fact was duly appreciated efforts were made to isolate the protective substances. With the same end in view, attention was directed to securing as strong a serum as possible. Thus in the early antitoxin days the serum contained only about 100 units per c.c. By employing selected horses and certain strains of bacilli to obtain strong toxin, such as the N. Y.

Health Department culture No. 8, a higher grade of serum was gradually obtained, so that after some years the ordinary serum was about 200 units per c.c. in some countries and in others 300 c.c. This of course required much smaller injections of serum to give the same dose of antitoxin. Nevertheless, even though the serum of many laboratories now runs as high as 400 to 500 units per c.c., rashes are still quite frequent. It has always been the hope of investigators to eliminate entirely the non-protective portion of the serum.

There have already been many attempts to accomplish this in the case of the antitoxins. Those interested in the chemical side of these investigations are referred to the recent article by Gibson. (*Jour. Biol. Chem.*, Vol. 1, No. 2.) In 1900, Atkinson, working in the Laboratory, eliminated all but the globulin from the antitoxic serum, and we tried this partially refined serum in 36 cases. The results were so nearly identical with an equal number of cases treated with the whole serum from the same horse that it did not seem to be worth while to go to the expense of preparing such an antitoxic solution. The idea that a practical separation of the antitoxin from much of the proteid non-antitoxic portion of the serum was possible was not given up. In August of 1905 we began trials with an antitoxic preparation which offered grounds for hoping for better success. Dr. R. B. Gibson, chemist in the Research Laboratory, placed the ammonium sulphate precipitate from the antitoxic serum in saturated sodium chloride solution and found that the globulin soluble in this contained all the antitoxin. In this way the nucleoproteids and the insoluble globulins present in the Atkinson preparation were eliminated. These soluble globulins were precipitated by acetic acid placed in a sac of parchment membrane and dialysed. This solution of globulins was then neutralized and to it sufficient sodium chloride was added.

This antitoxic solution containing that portion of the other soluble serum globulins which are soluble in salt solution was then tested. The results were from the start favorable, except that in the beginning more local pain was produced than with

the whole serum. Stricter attention to the neutralization soon overcame this, so that when the serum was injected on one side and the globulin solution on the other, the patient was unable to tell the one from the other. In October, 1905, the antitoxic globulin solution was administered not only in the hospitals but also in private homes by medical inspectors. Since December it has been gradually distributed throughout New York City and is now the only form of antitoxin supplied by the Health Department.

SERUM RESULTS FROM THE USE OF GLOBULIN SOLUTION.

The antitoxic effects seemed to be identical with that of the whole serum. This would be almost necessarily so, as the unit of the antitoxic globulin solution is identical with the unit of antitoxin supplied by the United States Government. Our tests appear to show that not only the toxin but also the poisons produced in the animal by injections with virulent bacilli are neutralized as completely by the globulin solution as by the antitoxic serum from which it is separated. Not only we ourselves but the resident and attending physicians of the contagious disease hospitals, noted that following the injections of the globulin solution there seemed to be decidedly less severe rashes than formerly followed the whole serum, and it was especially noted that there were very few who had any constitutional disturbances even when the development of the rashes did occur. As the serum supplied by different horses or from the same horse at different times is known to vary, and as it is therefore difficult to accurately compare different bleedings, it was decided to make a test by collecting a quantity of serum from four different horses, mixing it thoroughly, and then after precipitating one-half to treat an equal number simultaneously with the two preparations. These tests were chiefly carried out in the Willard Parker Hospital, but a few of the cases were treated at Riverside Hospital. To Drs. Lynah, Throne and Watson, the resident physicians in charge of these two hospitals, we are indebted for interest and aid in carrying out the experiment. It soon became evident that the serum that

we had chosen for the test was one of such character that eruptions and constitutional disturbances usually appeared in those injected. Whether it was the fact that serum from four horses had been mixed or whether it was some other reason, this serum produced more after-effects than any lot we had used in the hospitals since 1899. These after-effects were so marked and occurred in such a large proportion of the children that we decided to stop the use of the whole serum as soon as we became aware of the fact. In those over 10 years of age almost no rashes occurred. The rashes in those given the globulin preparation were much less severe. The cases treated with both the whole serum and the antitoxic globulins were most carefully watched, and the course of the disease as well as after-effects noted.

After all the tested cases had become fully convalescent or had left the hospital the histories were finally gone over and compared. It was found that fifty children under ten years of age treated with the whole serum had lived at least nine days, or long enough for the development of serum effects. The first fifty consecutive cases in children under 10 years treated with the antitoxic globulins precipitated from the same lot of serum were taken to compare with these.

Comparative table giving a summary of the constitutional and local reactions obtained in the treatment of fifty cases of diphtheria in young children, with a lot of antitoxic serum received from four horses and of an equal number of similar cases treated with a solution of the antitoxic globulins derived from a portion of the same lot of serum:—

	Children treated with the whole serum	Children who were treated with the antitoxic globulins
Marked constitutional symptoms accompanied by a severe and persistent rash in	28 per cent.	0 per cent.
Moderate constitutional symptoms accompanied by a well-developed erythema or urticaria in.....	18 per cent.	4 per cent.
Very slight constitutional disturbance accompanied by a more or less general rash in	20 per cent.	8 per cent.

	Children treated with the whole serum	Children who were treated with the antitoxic globulins
No appreciable constitutional disturbance, but a more or less general urticaria or erythema in	4 per cent.	34 per cent.
No appreciable deleterious after-affects whatever in..	30 per cent.	54 per cent.

DURATION OF RASHES.

Days	1	2	3	4	5	6	7	8	Totals
Antitoxic globulin cases.....	5	7	5	2	3		1		—23
Whole serum cases.....	1	4	10	1	10	3	2	5	—36

The concentration of antitoxin made possible by the elimination of the non-antitoxic substances is not only a convenience but is of distinct importance, as it tends to encourage large doses.

The antitoxic globulin solution tends to become slightly cloudy when kept at moderate or high temperatures and substances such as strong solutions of carbolic acid and trikresol precipitate it.

CALCIUM SALTS AS A PREVENTIVE OF SERUM SICKNESS.

Netter has recently published a clinical study in which the internal administration of calcium chloride is advanced as a preventive of serum rashes. On the day of the injection and the two following ones, he gives one gramme calcium chloride; if the quantity of serum injected is over 40 c.c. larger doses of the salt must be given, and over a longer period. He claims to have had excellent results.

We have had no experience with the method.

THEORIES TO EXPLAIN THE CAUSE OF SERUM SICKNESS.

The explanation of the serum rashes which was offered by Hamburger and Moro, was that the phenomenon was directly connected with precipitin formation, in fact, so directly related, that the exanthema was believed to be due to precipitin formation in the capillaries. This view, however, was abandoned after it was demonstrated that precipitin formation was only a test tube phenomenon and not one that occurred in the living body.

According to the researches of v. Pirquet and Schick rashes often develop before the precipitin can be demonstrated in the test tube and, on the other hand, precipitins may be abundant and yet no serum symptoms occur.

Nevertheless these authors believe that serum sickness is due to a chemical reaction between the horse serum and certain antibodies in the injected body. They are certain that these antibodies are not identical with the precipitins. They explain the long incubation period by regarding this as the interval necessary for the production of the antibody in response to the stimulation produced by the foreign serum. This antibody eventually reacts with some of the serum still present in the tissues. They point to the fact that a re-injection is associated with a shortened period of incubation and this, according to them, is strong evidence of the correctness of the above explanation. The incubation period is lessened because the cells can now produce the antibody more quickly. In addition to this "accelerated reaction" v. Pirquet and Schick cite cases of "immediate reactions." Such an immediate reaction may be produced during a period not earlier than twelve days after the primary injection and not much later than about nine months after. During this time they believe the injected body contains some free antibody which, when brought into contact with the exciting serum (a re-injection) produces at once symptoms similar to those produced originally. If the re-injection, however, is performed sooner than the twelfth-day period above mentioned, there is merely an "accelerated reaction," for there is, as yet, no free antibody in the injected body.

The immediate reaction seems to be comparable to the phenomenon described by Rosenau and Anderson, and by Otto. These authors showed that injections of very small amounts of horse serum sufficed to make guinea pigs extremely susceptible to subsequent injections of antitoxic or normal horse serum, provided the second injection occurs ten days or more after the first injection. Even the feeding of horse meat sensitized the guinea pigs.

While this seems to hold in guinea pigs it does not seem to

hold to the same extent for other animals, and certainly not in the case of man. Children have in many instances been injected with antidiphtheritic horse serum at intervals of two weeks without causing serious symptoms and never sudden death. The few cases of sudden death on record have all been after the first injection of serum.

In many hundreds of cases in institutions, cases of which we have thorough knowledge, a second immunizing injection of antitoxin after an interval of two or three weeks has not produced much more severe after-effects than the original injection. Some, however, have reported severe reactions with signs of collapse but no fatal results have yet been reported.

SUDDEN DEATH AFTER A SERUM INJECTION.

From time to time, but at long intervals, cases of sudden death after an injection of antitoxin have been reported. Some of these when investigated are clearly simply deaths from heart paralysis on account of diphtheria toxemia. A few cannot thus be explained, and in a few others the cause cannot be considered as settled.

In eleven years over fifty thousand cases of diphtheria have been injected with serum obtained from the N. Y. Health Department. From this large number only two cases of sudden death following the serum injection have been reported. In one family a child two years old, who was sick with diphtheria, received 5,000 units and a healthy sister of six years 1,000 units. The inspector who had injected them left in about ten minutes, no bad after-effects being noted. A few minutes later, according to the mother, the immunized child suddenly collapsed and in a few moments was dead. An autopsy revealed the fact that the child had the status lymphaticus strongly marked. The sister who received the larger injection from the same bottle had no bad after-effects and made a good recovery.

The other case was that of a lad sixteen years old who was suffering from a well-developed but not malignant pharyngeal diphtheria. He had been sick about twenty-four hours. He took the injection while in bed and did not show any

immediate reaction. The physician and nurse left the room for a moment. They heard a peculiar sound and quickly stepped back into the room and found the boy dead. This fatal ending may have been due to a heart weakened by diphtheria poison and not to the serum injection except in so far as that caused the exertion, or it may have been a case like that of the immunized child. No autopsy was performed.

The following case of sudden collapse has also come to our knowledge:

A young man with a moderately severe attack of diphtheria was injected with about 5 c.c. of serum. In about two minutes he felt a tingling sensation and then soon felt faint and as if sinking. Everything became black and he became unconscious. The physician found him with a very rapid heart action and cyanotic. He injected strychnine and carried out artificial respiration. In a few minutes he began to regain consciousness and in about half an hour returned to the condition present before the injection.

RESULTS OF ANTITOXIN TREATMENT IN DIPHTHERIA.

A period of more than ten years having elapsed since the introduction and general use of diphtheria antitoxin, sufficient time has passed to enable us to judge fairly accurately the effect this treatment has had on the mortality from diphtheria.

On looking over the literature one finds that such studies have been made a number of times, and that many of them are exceedingly valuable. Nevertheless, objections have been made, particularly by those not convinced of the value of serum treatment, that such statistics are open to grave sources of error, and that the lessened mortality and number of deaths is only temporary. Instead of this contention receiving support as the antitoxin treatment extends over a longer period and becomes more general, the trend is toward a lower mortality.

Before considering mortality statistics in cities let us note the results of antitoxin in operative cases both in hospitals and in private practice.

A comparison of the mortality in the operative cases is of particular interest, since these are always diphtherias of a severe type. The following figures are compiled from Lovett and Munro, Holt, McCollom and from our own official records:

OPERATIVE CASES Without Serum	Total Cases	Died	Mortality
German Authors	5795	3944	69 per cent.
German Hospitals	3063	2124	70 per cent.
British Authors	433	295	69 per cent.
French Authors	9242	6834	76 per cent.
American Authors—Pri- vate Practice	5625	3848	68 per cent.
Various countries	1993	1336	68 per cent.
Boston City Hospital (23 years)	71 per cent.

OPERATIVE CASES With Serum	Total Cases	Died	Mortality
Welch—European Hos- pitals	342	112	28.8 per cent.
Amer. Pediatric So. Re- port, Private Practice.	533	138	25.9 per cent.
McCollom—Hospital Cases—Boston	1553	683	44.0 per cent.
New York Health Dept. as below	1660	723	43.5 per cent.

Tenement service, 1895-7	144 cases	56 deaths
Tenement service, 1902-4	133 cases	39 deaths
Diphtheria Hospital, 1901-5	1341 cases	614 deaths
Outside Physicians, 1895-6	42 cases	14 deaths

Roux gives the mortality in operative cases as 46 per cent., as compared to 76 per cent. in preantitoxin times. With Ganghofner it is 13.6, as compared to 59.8-78 per cent. According to the Hungarian Health authorities it is 32.3 per cent., as compared to 50-70 per cent. formerly.

PROGNOSIS OF CASES INFLUENCED BY DAY OF BEGINNING

TREATMENT.

This subject is of importance because of its bearing on the evidence showing the necessity of the early administration of antitoxin, the statistics upon this subject being chiefly taken from hospitals.

There is no doubt that in general the earlier a case of diphtheria receives suitable care and surroundings, as in a hospital, the better its chance of recovery, irrespective of any specific method of treatment. This fact, which was made use of by those who denied the efficacy of serum treatment, is well shown by the following table, published by Glaser, and dealing with 619 children treated in a hospital without antitoxin:

Admission on the	
2nd day of the disease.	Mortality.....19 per cent.
3rd day of the disease.	Mortality.....24 per cent.
4th day of the disease.	Mortality.....38 per cent.
5th day of the disease.	Mortality.....30 per cent.
6th day of the disease.	Mortality.....21 per cent.
7th day of the disease.	Mortality.....41 per cent.

Here we see that the increase in mortality is quite distinct though somewhat irregular. It is merely necessary, however, to compare these percentages with similarly arranged statistics of cases treated with antitoxin to see at once the great value of the early administration of antitoxin.

Bing and v. Ellerman (*Therap. Monatshefte*, 1904, xviii, p. 398) have critically studied a large number of cases as to the reason of the greater mortality in cases received after the second day. They cite Heubner, who had caused the prognosis to be written opposite each history on the admission of the patient. The results showed that the patients admitted later were more apt to die mainly because they were admitted in worse condition than the others. Heubner's results may be tabulated as follows:

Admission on the	Unfavorable Prognosis % of Cases.
1st day	6
2nd day	8
3rd day	14
4th day	17
5th day	22
6th or 7th days	53
8th and later	69

Bing and v. Ellerman then give their own report on 1,356 cases of diphtheria from the preantitoxin days (1889-1894), occurring in the Blegdams Hospital in Copenhagen.

Day of Admission	Number Admitted	Deaths	Mortality Per cent.
1	113	38	34
2	494	110	22
3	350	95	27
4	177	68	38
5	125	53	42
6	54	22	41
7	23	13	57
8 and later	20	12	60

They say:

“If the mortality is calculated for periods of 48 hours instead of 24 hours the irregularities disappear and one obtains the series 24, 31, 42, 58%.”

THE VALUE OF ANTITOXIN TREATMENT INFLUENCED BY DAY OF DISEASE WHEN IT IS ADMINISTERED.

Two examples of this will suffice. Faber tabulated the results in 3,167 cases of diphtheria treated in the Blegdams Hospital, Copenhagen. He excludes cases complicated with scarlet-fever, whooping cough and other diseases unless these had passed their height and were receding. These tables should be compared with those just given from the preantitoxin period.

Commencement of serum treatment	No. of patients	No. of deaths	Mortality per cent.	Calculated No. of deaths according to the entire mortality of the group 11.5%	Difference between actual and calculated mortalities
1st day	99	7	7.1	11	— 4
2nd day	641	48	7.5	74	—26
3rd day	763	69	9.0	88	—19
4th day	555	63	11.4	64	— 1
5th day	334	52	15.6	38	+14
6th day	171	29	17.0	20	+ 9
7th day	80	17	21.3	8	+ 8
Later than 7th..	196	39	19.9	23	+16
Unknown	298	35
Totals	3137	359	Av. 11.5	—	—

Cohn gives the following figures for 1,000 cases of diphtheria treated in the Moabit Hospital, Berlin:

Treatment begun on 1st day	78 cases	1 died =	1.3%
Treatment begun on 2nd day	361 cases	40 died =	11.1%
Treatment begun on 3rd day	284 cases	30 died =	10.5%
Treatment begun on 4th day	101 cases	25 died =	24.7%
Later or undetermined	176 cases	40 died =	22.7%

Similar statistics have been published by Heubner, Aaser, Frank, and Jellineck. The mortality increased from the first day on, from about 4 or 5% up to 20% or more.

REGARDING A POSSIBLE DECLINE IN VIRULENCE OF THE DISEASE IN RECENT TIME.

It has been urged, especially by opponents of serum therapy, that the disease itself is becoming milder, entirely aside from the use of diphtheria antitoxin. Careful study of the subject fails to bear out this contention, for among those who do not receive antitoxin the mortality at the present day is still as high as ever. This question has recently been studied especially by Zucker (*Wiener Klin. Wochenschrift*, No. 44, 1905), who found among the rural population in his province abundant statistics on cases not treated with antitoxin. Thus in Steiermark, at the most two-thirds of the cases received antitoxin. The total number of cases which did not receive antitoxin (since the introduction of the same) was 12,000. His chart shows very well the difference in mortality between those cases receiving antitoxin and those not receiving it. The mortality in the cases treated with antitoxin varied from 11 and 15 per cent., while those not treated had a mortality of from 32 to 42 per cent.

Zucker gives analogous figures for non-antitoxin cases from the statistics of Graz. In these the case mortality was 21.7 per cent. in the quinquennium 1894-98 inclusive, and 22.6 per cent. in the quinquennium 1899-1903 inclusive.

An absolutely ideal method to show the influence of antitoxin is one made use of by Fibinger (cited by Faber, *Jahrb. f. Kin-*

derheilk., 1904, No. 59). In this, at the same time every other case was treated with antitoxin, he had

239 cases with Antitoxin-mortality of 8.....	3%
245 cases without Antitoxin-mortality of 30.....	12%

This method, however, for obvious reasons is not available at this day. We once made a similar test at the Willard Parker Hospital. The difference in the behavior of the cases was so greatly in favor of the antitoxin that the test was stopped and all cases put on antitoxin.

Perhaps the least objectionable of all methods at present available is a comparison of the absolute number of deaths per 100,000 for a long period of years before and after the introduction of antitoxin. Such figures, moreover, must be collected only from such cities where reliable statistics have been kept during the entire period and any extraneous factors, such as changes in the nomenclature, the presence of epidemics, must be known.

ABSOLUTE MORTALITIES IN LARGE CITIES, PROVIDED LARGE SERIES
OF YEARS SHOWN, ARE RELIABLE INDEX OF THE VALUE
OF ANTITOXIN TREATMENT.

It was stated above that the statistics must be from a long period of years. While this of course is true for all kinds of statistics, it is particularly important in diphtheria in which mortality figures move up and down irregularly in large waves. These irregularities, however, only become apparent when a considerable number of years is gone over. To give an example: In Baltimore, in the six years ending 1882 the average of deaths per 100,000 from diphtheria and croup was always above 140 and reached 200 or over in three of those years. In the seven years following the mortality fell sharply and continuously until it reached its ebb point in 1889, when it was 52 per 100,000, and yet no difference in treatment occurred in 1882.

One must, therefore, be careful not merely to take readings which constitute part of an epidemic, unless due allowance be

made for this fact. And in order to appreciate what an epidemic is, one must know the average number of deaths for many years back.

Statistics of this kind ought furthermore to be taken mainly from the large cities, for reports of deaths are usually but indifferently kept in the rural districts. Thus in some states the cause of death was often certified to the health authorities by the town supervisor, so that it happened that "sore inside," "chronic," "running sores," were occasionally given as causes of death.

In the following pages statistics have been collected from a number of large cities on the continent of Europe, in Great Britain and in the United States. In all of these cities the registration of deaths has long been very efficient.

For purposes of comparison it will be perfectly fair to take the combined mortality from diphtheria and croup for the years of the preantitoxin period and compare this with that of the years since the introduction of antitoxin.

Combined statistics, deaths and death rates from diphtheria and croup.—New York, Brooklyn, Boston, Pittsburg, Baltimore, Philadelphia, Berlin, Cologne, Breslau, Dresden, Hamburg, Königsberg, Munich, Vienna, London, Glasgow, Liverpool, Paris, Frankfurt.

Year	Population	Deaths Diphtheria and Croup	Rate per 100,000
1878	10,000,598	8,185	81.8
1879	10,188,268	7,205	70.7
1880	13,401,394	11,526	86.0
1881	13,642,366	13,897	101.9
1882	13,857,726	14,075	101.6
1883	14,049,727	13,721	97.6
1884	14,353,102	11,930	83.2
1885	14,544,489	12,399	85.2
1886	15,617,867	12,385	80.8
1887	16,217,823	12,721	79.5
1888	16,300,948	11,798	73.7
1889	16,526,135	13,247	75.1
1890	16,526,135	11,059	66.9
1891	17,689,146	12,389	70.0
1892	18,330,737	14,200	77.5
1893	18,467,970	15,726	80.4

Year	Population	Deaths Diphtheria and Croup	Rate per 100,000
1894	19,033,902	15,125	79.9
1895	19,143,188	10,657	55.6
1896	19,489,683	9,651	49.5
1897	19,800,629	8,942	45.2
1898	20,037,918	7,170	35.7
1899	20,358,857	7,256	35.6
1900	20,764,614	6,791	32.7
1901	20,874,572	6,104	29.2
1902	21,552,398	5,630	26.1
1903	21,865,299	3,117	23.4
1904	22,532,848	4,917	21.8
1905	22,790,000	4,323	19.0

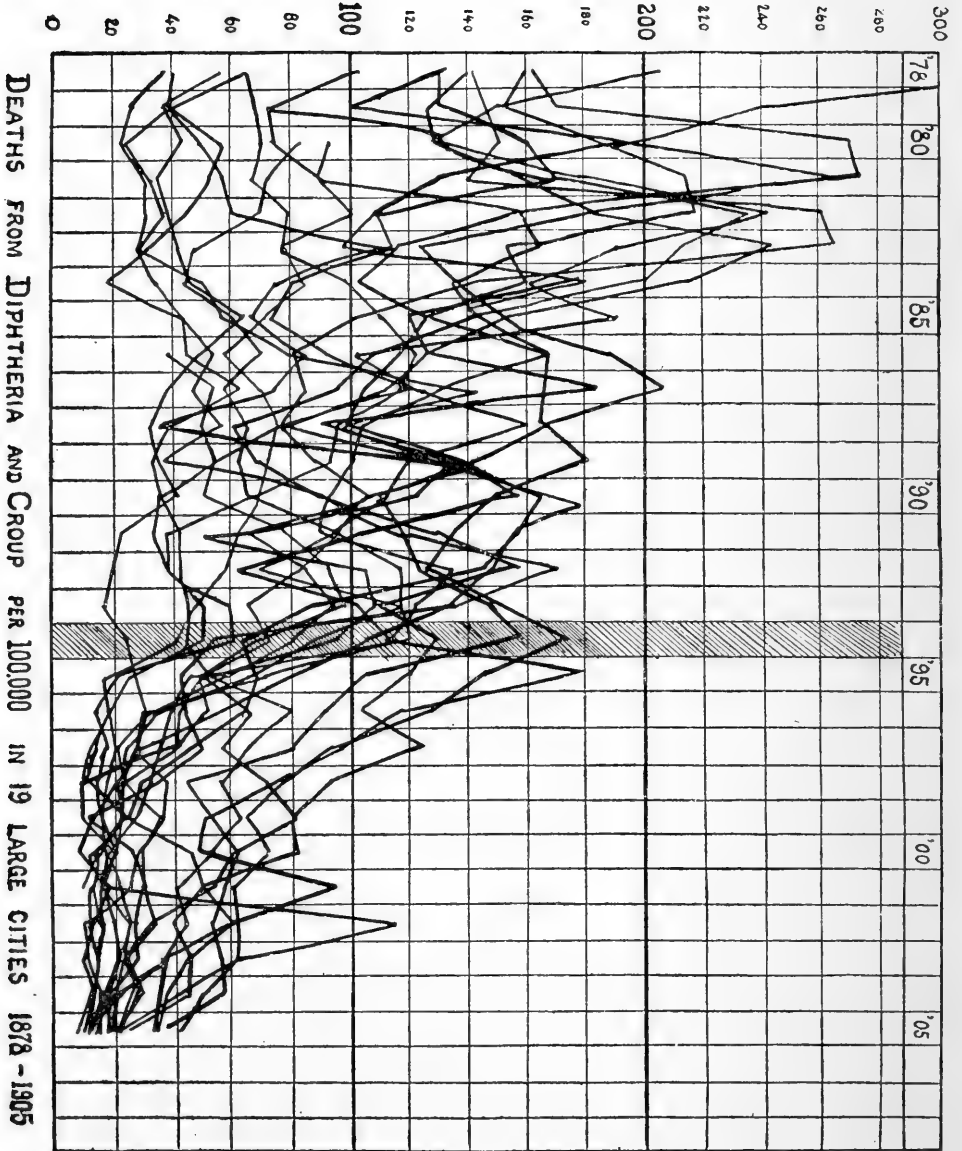
The statistics for Vienna do not begin until 1880, those for Glasgow until 1886. The figures for Paris are those of diphtheria only.

These figures are much more striking if arranged in the form of a curve as seen on the following page.

Here we see that although in the preantitoxin years there were marked fluctuations in the absolute mortality per 100,000, there is no period in which all of the cities show a decrease. Thus in 1884 about half of the cities show a decline and the other half an increase in mortality; the same is true for 1888. Not until we come to the critical year 1894 do we find that almost all the cities show a like behavior. And this drop has continued until the present time.

Two rather sharp rises in the antitoxin years, one in 1901 and the other in 1902, attract our attention. The first of these was in Boston, where a moderate epidemic occurred. In the course of the next two years, however, the disease had again come to its low point.

The other sharp rise in 1902 was in Königsberg and is particularly instructive. In 1901 there had been 16 diphtheria deaths per 100,000 population, in 1902 there were 115, and in the following three years 61, 42, 22 respectively. Here we see that the disease assuming epidemic proportions in 1902 was not entirely checked until after three years had elapsed. In a discussion on this subject in Königsberg, 1903, all the speakers agreed that the reason for the spread of the disease was the



failure to use immunizing injections. The city authorities did not supply the serum free and the patients usually objected to the expense. In consequence of this but very few immunizations were carried out. (See *D. med. Wochenschrift, Vereinsbeilage*, No. 10, Mar. 5, 1903.)

The following statistics from New York city are very striking.

Table showing number of cases, deaths and mortality per cent. of diphtheria in the boroughs of Manhattan and the Bronx from 1893 to 1905, inclusive:—

Period	Cases	Deaths	Mortality per cent.	Death rate per 100,000
1893	7,021	2,558	36.4	145.3
1894	9,641	2,870	29.7	158.6
1895	10,353	1,976	19.1	105.6
1896	11,399	1,763	15.4	92.5
1897	10,896	1,590	14.6	81.9
1898	7,593	923	12.2	46.7
1899	8,240	1,087	13.1	53.9
1900	8,364	1,121	13.4	54.5
1901	7,726	1,227	15.9	57.9
1902	10,429	1,142	10.9	52.3
1903	11,662	1,302	11.2	57.9
1904	12,517	1,311	10.5	56.5
1905	8,541	860	10.	36.0

The decrease in the death rate from 158 to 36 since antitoxin was first introduced into general use by the Department, January 1, 1895, is certainly a wonderful reduction.

CLINICAL EFFECTS OF DIPHTHERIA ANTITOXIN.

The clinical effects of diphtheria antitoxin manifest themselves both on the toxemia of the disease and on the pseudomembrane.

In cases treated early the general condition of the patient becomes noticeably bettered, the constitutional symptoms of toxemia disappear, the color and appearance of the patient improves, the appetite returns and mental depression disappears. When toxemia has become well marked on account of late treatment and cellular poisoning is manifest, the above effects are much less noticeable, or take place more gradually.

In favorable cases, and especially in uncomplicated diphtheria the temperature is not as a rule high, and after an injection falls rapidly, becoming practically normal in three or four days. This, however, often happens even without antitoxin. In cases of mixed infection and also in laryngeal cases with some bronchial involvement, the drop in temperature is much less sudden and takes place by lysis. When the temperature does not fall in the regular way, it is usually an indication of an otitis, a pneumonia, or of some other complication. Upon bronchopneumonia, even when partly due to diphtheria bacilli, antitoxin has but little effect.

The local effects produced by the absorbed antitoxic serum on the recently produced pseudomembrane are marked. In early cases of diphtheria of the pharynx or tonsils, six to twelve hours after a sufficiently large injection the pseudomembrane ceases to extend and becomes blanched, the dirty color, if present, being less marked; the membrane appears to swell, becoming whiter and seemingly thicker. Later the membrane becomes loosened at the edges and rolled up and soon detaches itself, either en masse or in small pieces, spontaneously, or following irrigation. The process takes place somewhat more rapidly on the tonsils than elsewhere in the pharynx. The time required for the complete restoration of the mucous membrane in early treated and favorably progressing cases when epithelial necrosis has not been extensive, varies from twenty-four hours to three or four days. Occasionally the pseudomembrane, after disappearing, will be partially reproduced, in which case a second dose of serum will cause it to undergo the changes mentioned above. In the cases of diphtheria in which the pyogenic streptococci or other bacteria play an important part, the effects of the antitoxin are not so well marked and the exudate or pseudomembrane persists for some time. When the lesions have lasted for some days and the superficial epithelium has become necrotic, there remains for several days after separation of the membrane an ulceration which is coated by a thin exudate. A week or ten days will be required in these cases for complete local recovery.

In nasal diphtheria, after inoculation, the effects are similar, the irrigation, if practiced, bringing away small or large pieces of detached membrane, or sometimes a complete cast of the cavity. The nasal discharge and swelling are usually soon ameliorated and mouth-breathing ceases. In laryngeal diphtheria, when given early, the serum checks the process so that in many of the cases operation does not become necessary. If intubation or tracheotomy has to be practiced, the membrane is soon loosened in favorable cases and is coughed up. The shorter average time for which the tube is required in intubation and the canula in tracheotomy has been noted by many. It is a notable fact that the intubation tube is more often coughed out with the use of antitoxin than in any other form of treatment.

ATTEMPTS TO PRODUCE A SERUM DIRECTED PARTICULARLY AGAINST THE LIFE OF THE DIPHTHERIA BACILLI.

No experiments have been reported which prove that a serum is attainable which will have any value when injected to directly act upon and destroy the diphtheria bacilli. We have made a serious attempt to produce such a serum but without any success. Wasserman, Martin and others have produced a serum which when applied to the bacilli in a test tube has an agglutinating and perhaps a slight bactericidal effect.

This serum has been applied locally in cases of diphtheria and it is claimed to aid the disappearance of the bacilli from the throat and nostrils.

Dopter, as well as Vogelsberger, reports favorably on its use. Our limited experience with such a serum was disappointing, as no results were noticeable.

DURATION OF IMMUNITY.

It was early recognized that diphtheria antitoxin could be employed in immunizing those exposed to diphtheria infection. This use of the serum was advocated especially in France and in the United States. Thus in 1896 Biggs and Guerard were able to collect statistics of over 17,000 persons injected with doses of from 500 to 1,000 units. The majority of these

were in contact with diphtheria. Out of this large number only 131 were attacked with the disease, 110 within 30 days and 21 after that time. Of these 131 only two died.

Netter (*Presse Médicale*, 1902) has collected the results of 34,350 immunizing injections. Despite the injections 206 (= 0.6%) of the cases developed diphtheria. Most of the observations deal with orphan asylums, schools, foundling asylums and the like.

Experience has proved that complete immunity is secured for only a short time—two to four weeks would probably be the usual duration—but in some it is complete for only ten days to two weeks. At the Eleventh Congress for Hygiene and Dermography, Brussels, 1903, practically all the observers agreed that the duration of passive immunity is in most cases about three weeks. The injection of even large amounts of serum is unable to greatly prolong this period. This is not to be wondered at, for we know from pre-serum days that second attacks of diphtheria were now and then observed within four to six weeks after the primary attack and here we have to deal with the more lasting active immunity.

Ibrahim has recently published a study on a large number of cases treated with antitoxin. Out of these he mentions eight in which second attacks occurred after 20, 21, 25, 31, 40, 41, 43, and 59 days respectively. He counted as a relapse only such throats as had a typical whitish membrane and in which all the clinical symptoms of diphtheria were present.

This point is important because so-called secondary membranes are frequently observed on the eighth to the twelfth day. These are usually yellowish, easily rubbed off, and the patients lack the other clinical evidences of diphtheria.

In view of the short duration of passive immunity it is advisable to repeat every three weeks immunizing injections in persons exposed to diphtheria for a long time.

REPEATED INJECTIONS OF ANTITOXIN.

In view of the recent studies in immunity a number of observers have suggested that the injection of diphtheria anti-

toxin might be followed by the production of an antibody against this. Such a phenomenon, however, is never observed in the treatment of diphtheria as there is not time for the development of such a substance.

DOSAGE OF ANTITOXIN.

The average amount of antitoxin used per case is gradually becoming greater. Two to five thousand units are given in light or moderately severe cases and ten to thirty thousand units in very severe cases.

INTRAVENOUS INJECTIONS.

Antitoxin when intravenously injected reaches the tissues more quickly and in greater concentration than when given by the usual subcutaneous method. A number of hundred cases have been treated in this way with favorable results. It is to be recommended in very severe cases.

THE ANTITOXIC TREATMENT OF TETANUS.

In a case of tetanus the bacilli remain localized at the point of inoculation and multiply comparatively little. Toxins there produced are absorbed very slowly. One of the poisons called tetanolysin acts upon the blood corpuscles, while the other and more important, the tetanospasmin, has a peculiar affinity for the nervous and muscular system, and exerts its poisonous action almost wholly upon the nerve and muscle cells. Recent investigations seem to show that the tetanospasmin passes to the central nervous system largely if not wholly by the motor nerves, which take up the toxins through the intramuscular nerve endings. From the infected wound the toxins partly pass into the nerve endings and muscle cells adjacent to them and partly into the blood. From the blood and tissue fluids the toxins are taken up by the nerve endings throughout the body. Tetanus antitoxin is not taken up by the nerve endings. It follows therefore that tetanus antitoxin not only cannot neutralize the toxins already attached to the nerve cells but probably also that portion which has entered the nerve fibres. Only the toxins which are free in the blood and tissues can be

certainly neutralized. With these facts in mind it is plain why the serum treatment of tetanus accomplishes so much less than that of diphtheria. We diagnose diphtheria by the exudate in the throat before serious poisoning has taken place. We diagnose tetanus by the symptoms which follow after the union of the toxins with the cells as well as its absorption by the nerves. Tetanus antitoxin can only save life when it can be given before enough poison has been absorbed to cause death. Undoubtedly we meet many cases too late to expect anything from antitoxin. Here absolutely no effects are noted after even enormous injections. I believe it is such cases as well as experimental ones in animals to which there was given either too small an amount of antitoxin or too great an amount of infection that have given rise to the pessimistic views of many as to the value of tetanus antitoxin in cases of tetanus. As to the immunizing value of tetanus antitoxin there is unanimity of opinion. The clearest evidence has come from the immunization of horses. In our own antitoxin stables we used to lose several horses almost every year until we immunized all the animals every three months with 50 c.c. of antitoxic serum. Similar results have followed this practice in other stables. It is the custom at many dispensaries in New York city and elsewhere to immunize all Fourth of July wounds by injecting 10 c.c. of serum. None of these have ever developed tetanus. Even the eleven cases of human tetanus which have been reported as happening in Europe after single injections of antitoxin have given proof of the value of immunizing injections, for the mortality was only 27 per cent. These cases teach also that in cases where tetanus infection is suspected the antitoxic serum should be given a second and sometimes even a third time, at intervals of seven days. The opinions of different physicians as to the value of the antitoxic treatment of developed tetanus vary widely. The disease runs such a different course in different persons and is so comparatively rare that it is difficult to judge by either statistics or personal experience. It is interesting to note that the two latest authoritative reviews by American writers differ greatly

in their conclusions. Anders and Morgan state, "The present status of the serum question leaves no room for doubting that when given during a well-developed case of tetanus, antitoxin does not have any appreciable beneficial effect, neither the mortality being reduced nor recovery hastened thereby."

McFarland states as his opinion, "It would seem, therefore, that we have in tetanus antitoxin not a specific, because it fails too often to have merited that name, but a valuable remedy in the treatment of the disease, and one that ought not to be neglected until a better remedy is supplied." My own opinion founded on reading and personal experience agrees with that of McFarland. I have seen cases of generalized tetanus that after a large intravenous injection have markedly improved, and finally recovered and these cases have certainly done better on the average than apparently similar cases getting palliative treatment alone.

In the endeavor to obtain more frequent curative results the antitoxin has been injected into the ventricles of the brain, and recently by the advice of Rogers, of New York city, into the neural sheaths and into the substance of the spinal cord. Both on theoretical grounds and clinical results the injection of antitoxin into the ventricles has been given up. In coöperation with Dr. Cyrus W. Field I have recently tried a number of experiments upon guinea pigs, to test the importance of intravenous and of intraneural injections of antitoxin in animals in which tetanus had already developed. Up to the present time over forty guinea pigs have been experimented upon. These were infected in the lower part of the hind leg with ten to twenty times the fatal dose of tetanus toxin. Within from one to two hours after the development of the first definite symptoms of tetanus the animals were operated upon and given antitoxin. The experiments show clearly that moderate doses of antitoxin given after the development of tetanus did not save the animals from death or even prolong life, while very large doses usually did both. Seventy-five per cent. of those receiving 5 c.c. of serum recovered. The surprising result developed that amputation of the infected leg at the hip-joint hastened the death of the

animals in every case. Control animals which had not been infected stood the amputation perfectly well and made good recoveries. Without antitoxin, excision of a piece of the nerve did not materially prolong life, nor did ligation of the nerve. In the guinea pigs receiving antitoxin the ligation of the nerve seemed to be of benefit. The results of the experiments showed that large doses of antitoxin given shortly after the development of tetanus usually saved the animals and that most of the toxin was absorbed by the blood and not by the nerves of the infected part. Every minute of delay after the appearance of tetanus was of importance. I feel convinced that in human tetanus the most important thing is to give at the earliest possible moment after diagnosis a very large intravenous injection of antitoxin. From 50 to 75 c.c. of the most potent serum obtainable should be given. During succeeding days injections can be given either intravenously or subcutaneously until marked improvement or death has taken place. If a surgeon is at hand intraneural injections into the nerves supplying the infected portion of the body may also be given, but these, I believe, are not usually necessary if the large intravenous injections have been given. My own experience with these large intravenous injections has been favorable.

THE SERUM TREATMENT OF BACTERIAL DISEASES IN WHICH POISON-
ING IS DUE TO ENDOTOXINS RATHER THAN TO EXTRA-
CELLULAR TOXINS.

In the earlier part of this lecture we reviewed the difficulties of combating endotoxins.

The diseases excited by bacteria which produce chiefly endotoxins include all the important bacterial infections with the exception of diphtheria and tetanus.

In none of these diseases is the serum treatment at present markedly beneficial and in none have statistics as yet been able to decide as to whether the serum has done any appreciable good. It is true that enthusiastic reports have been made of the results in many of the infections, but these have been counteracted by negative reports from further trials. Horses

injected with most of the varieties of bacteria develop protective bodies, so that their serum is able to immunize animals to a considerable extent, but even when recently obtained it is not able to cure disease already fully established. My personal experience has been limited to the use of sera in dysentery, typhoid fever, pneumonia, and streptococcus infections. In dysentery only did I think I saw any curative results. It is perfectly possible that with sera of greater potency the results might have been a little more favorable. The time allotted to the lecture will allow of merely a brief statement as to the results of the use of sera in the most important diseases in which it has been tried.

THE SERUM TREATMENT OF BUBONIC PLAGUE.

It was found that repeated injection at first of dead plague bacilli, and later of living cultures, stimulated the cells of the horse to produce both antitoxic and bactericidal substances. This serum afforded protection to small animals inoculated with virulent cultures even if the serum was not given until twelve hours after infection. The first reports by Yersin were quite favorable. He noted that in the cases treated on the first day of the disease the fever, and all alarming symptoms, frequently quickly disappeared. In cases treated at a later period very large doses of 100 to 200 c.c. were required, and the beneficial results were not so manifest. The reports from the trials made throughout India which have recently been compiled do not give us as favorable an opinion. In the cases which were controlled by giving alternate cases the serum there was no decided difference in the death rate between the treated and the untreated cases. Most of the physicians, however, believed that the patients receiving the serum felt better after it.

THE SERUM TREATMENT OF BACILLARY DYSENTERY.

Dysentery offers some hopeful reasons why a serum treatment might be beneficial. The bacteria that excite it are in the great majority of cases either the bacillus identified by Shiga or several varieties of bacilli closely allied to it. The infection

is usually easily diagnosticated at its onset by its striking symptoms, and is limited to the mucous membranes of a portion of the intestines. The reports of the results from the use of serum have been favorable, although in no sense striking. My own experience in observing the treatment in twenty cases indicated to me that in typical cases with blood and mucus mixed with the discharges, the serum did unmistakable good. In simple diarrhœa it had no appreciable effect. In severe cases it is advised that the serum be given every day in 10 to 30 c.c. doses until convalescence. The patients showed less constitutional poisoning after its use and the blood and mucus rapidly diminished. The cases were too few to give statistics of any value.

THE SERUM TREATMENT OF TYPHOID FEVER.

When infection of the typhoid bacillus has advanced far enough to produce the symptoms of typhoid fever very extensive bacterial invasion has occurred. With our present knowledge we would not be very hopeful of beneficial results from injections of serum. With the exception of Chantemesse and his pupils in Paris no persons claim at present to have an anti-typhoid serum which has any marked effects. Chantemesse claims that in 507 cases treated with serum injections there were only 6 per cent. of deaths, while in the cases not treated there was a mortality of 12 per cent. The serum showed its good effects most clearly in the lighter cases. In these within a few hours moderate subjective improvement was felt. After 36 to 48 hours the reaction begins. After five to seven days the general condition is almost normal, but the rash may remain and the spleen continue enlarged. Outside of Paris but few trials of the serum have been made.

SERUM TREATMENT OF STREPTOCOCCUS INFECTIONS.

To estimate the present and future value of antistreptococcus serum is a matter of the utmost difficulty. Many of the cases reported are of little or no help, because no cultures having been made, we are in doubt as to the nature of the bacterial infection.

Marmorek's early results are the most favorable reported, but without casting any doubt upon the justification of his conclusions, from the data at his command, I believe they undoubtedly give too favorable a view of the value of the serum.

In the few cases of puerperal fever, erysipelas, wound infection, scarlet fever, and bronchopneumonia that I have seen under serum treatment the apparent results under the treatment have not been uniform or striking. Only occasionally have we seen results that were distinctly due to the serum.

In a number of cases of septicemia where chills had occurred daily they ceased absolutely or lessened under daily doses of 20 to 50 c.c. The temperature, though ceasing to rise to such heights, did not average more than one or two degrees lower than before the injections. In some cases of ulcerative endocarditis the serum treatment was kept up for many weeks without doing any permanent good. Some cases convalesced; others after a week or more grew worse and died. In some cases the temperature fell immediately upon giving the first injection of serum, and after subsequent injections remained normal, and the cases seemed greatly benefited. As a rule, in these cases no streptococci or any other organisms were obtained from the blood. In one case streptococci persisted for three months in spite of serum injections. In bronchopneumonia, laryngeal diphtheria, scarlet fever, smallpox, and phthisis, we have seen absolutely no effect. In the exanthemata our injections were much smaller than those used in Vienna, in which city very striking results are reported from 100 c.c. doses in cases of malignant scarlet fever as seen in the following quotation from Escherich:

"When large doses of antistreptococcic serum are given in scarlet fever the pulse and respiration fall and the rash fades. The most striking change is the improvement in the general condition. The delirium or drowsiness disappears, the appetite reappears and restlessness diminishes. Upon the local gangrenous processes there is no apparent effect. The mortality sank from an average of about 13 to one of 8 during the years 1901 and 1902 under the serum treatment."

Escherich stated in 1904 that the antistreptococcic horse serum was not of much value in light cases, but was of great value in those cases having a high temperature or which were of a fulminating character.

The results obtained here in New York by both physicians and surgeons have not, on the whole, been very encouraging in streptococcic infections. In some of the cases where apparently favorable results were obtained other bacteria than streptococci were found to be the cause of the disease.

A single antistreptococcic serum protects healthy rabbits from infection from most of the streptococci obtained from human sepsis, but not from all. Failure to do good in human infection cannot as a rule be attributed to the variety of streptococcus, as polyvalent sera have not given any uniformly better results. The serum will in animals limit an infection already started, if it has not progressed too far. The apparent therapeutic results in cases of human streptococcus infection are variable. In some cases the disease has undoubtedly advanced in spite of large injections, and here it has not seemed to have had any effect. In other cases good observers rightly or wrongly believe they have noticed great improvement from it. Except rashes, few have noticed deleterious results, although very large doses have been followed in several instances for a short time by albuminous urine and even temporary suppression.

In suitable cases we are warranted, I believe, in trying it, but we should not expect very striking results.

ACUTE ARTICULAR RHEUMATISM.

Many of the irregular cases of acute articular rheumatism are certainly due to streptococci and diplococci of various kinds. It is not improbable that all cases are excited by bacterial poisons. Acting upon this supposition a considerable number of persons have been injected with antistreptococcic serum. The results have been apparently favorable in some cases and doubtful in others. Further tests are necessary before final decisions can be made as to whether it is really beneficial.

GONORRHOEAL RHEUMATISM.

As is well known serum treatment of acute gonorrhœa has not as yet been proven to be of any value. It seems that upon the joint inflammation a serum may have marked effects. Rogers has just reported a number of cases treated by him with a serum prepared by Torrey in which some seemed to show unmistakable benefit. Upon the acute cases the results were frequently very striking while in the chronic the benefit was uncertain or absent. It is probably the cases in which few or no living gonococci are present in the joint that respond the best to the serum. If further tests confirm these first results they will stimulate attempts to overcome the difficulties in acute articular rheumatism.

CEREBROSPINAL MENINGITIS.

The results of serum treatment in man in infections due to the meningococcus have as yet been negative. For a time treatment with diphtheria antitoxin was employed by many. This had no effect whatever. Animal experiments have shown that a feebly antitoxic serum can be prepared.

PNEUMONIA.

As there are a number of distinct varieties of pneumococci a polyvalent serum which is fresh should alone be used. The doses should be repeated at intervals of 12 to 24 hours. The size of the dose is not agreed upon.

The latest report upon the treatment of lobar pneumonia is that of Winkelmann of Cologne published January 2nd. He treated 16 severe cases with doses of 10 or 20 c.c. He states that the serum was apparently harmless. Curative effect was certainly not marked, but appeared clear in a few of the cases. He believes it advisable to use it in severe cases. The duration of the disease was not shortened. The leucocyte count was not appreciably affected. My own experience has been limited to the use of a monovalent serum. Dr. Lambert, who prepared the serum in the Health Department Laboratory and cared

for the cases clinically, considered that the serum had some beneficial effect and probably prevented the development of a pneumococci septicemia. This was not always the case, and we finally stopped producing the serum. I think Winkelmann states the present state of serum therapy in pneumonia fairly. The serum, through its opsonins or other bodies, can be considered to prepare the bacteria for the attacks of the leucocytes and other cells.

TUBERCULOSIS.

At present there are no facts to demonstrate that a serum has been obtained which possesses distinct curative value in tuberculosis. Maragliano claims that a serum obtained by him has value and that it can be administered not only subcutaneously but also by the mouth. He injects 1 c.c. every other day and adds at times an injection of vaccine. Those who have observed his cases have remained in doubt as to the curative value of the serum. There can be no doubt that injections of the new tuberculin and of non-virulent bacilli raise the bactericidal powers of the serum and tissues so that we need not give up hope of obtaining a serum therapy of some value.

THE LOCAL APPLICATION OF ANTITOXIC SERUM IN HAY FEVER.

The hay fever toxins belong to the toxalbumins and are resistant to heat and acids but injured by alkalies. They are precipitated in saturated solution of ammonium sulphate.

Different persons vary greatly in their reaction to the toxins from the different plants. Some react to some and not to others, while some react to many. In this country the toxins in the rag weed and golden rod are most in evidence. A serum is obtained from horses, which are sensitive to the poison and are injected for several months with the toxins. After three months the horses possess considerable antitoxin. The toxin is mixed with antitoxin and the amount required to neutralize is determined by testing a sensitive conjunctiva.

The serum preserved in $\frac{1}{4}$ per cent. carbolic acid is dropped into the eyes and nasal cavities on arising and several times

during the day; in developed cases much more often. The dried serum powder is also snuffed up into the nostrils. The serum is not given subcutaneously, as it is less efficacious and is irritating. During the night patients protect themselves by sleeping with closed windows, as otherwise the serum is not sufficient to keep them comfortable. Lubbert reports (*Ther. Monat.*, Dec., 1904) in 505 European cases carefully recorded 59 per cent. greatly benefited, 28 per cent. doubtful and 13 per cent. failures. Most of the latter were due to improper application. Knight reports that in 200 cases in America about 50 per cent. were greatly benefited. That the serum made from injecting the proper toxins is serviceable there seems to be no doubt. In some cases equally good results are obtained from the use of adrenalin or other therapeutic measures. In some persons relief is obtained from the serum when other measures fail. Serum from grass-eating animals appears to normally contain antitoxic substances, and may be used therapeutically.

ANTITHYROID SERUM.

Lepine was one of the first to seriously attempt to immunize animals. He found that the serum of the immunized goat produces no ill effects on a healthy dog, if the dose is limited to 20 centigrammes, and a few days are allowed to elapse between doses; this amount seems preceptibly to diminish the thyroid function. Hence it seems legitimate to try an antithyroid serum in Basedow's disease, but the extreme susceptibility of sufferers to therapeutic measures of any description dictates great caution. Most striking results have been obtained by Beebe and Rogers. They have produced a serum by injecting into rabbits the extracted nucleoproteids and globulins of human thyroids. The thyroids of those suffering from Graves' disease gave the best materials. These results have been very encouraging and it seems certain that some cases which no other treatment benefited were greatly helped. Rogers in a very recent paper stated:

"I do not believe that a serum made by this method will cure all cases of the disease, as there have been very marked

differences in the individual reactions. As a general rule, although it is not invariable, those who have had the most reaction have progressed the most satisfactorily, and it does not seem to hasten recovery if the injections are constantly pushed. On the contrary, this seems to retard the progress.

“Then there is a certain type of cases which have seemed peculiarly refractory and liable to relapse. These patients have no exophthalmos but they do have an asymmetrical thyroid, that is, one with a cystic or edematous enlargement. They complain chiefly of digestive disturbance with fermentation and pain which may be abdominal or thoracic, and with this disturbance there is generally dyspnea and labored breathing. They also have marked weakness, a soft feeble pulse and either an intermittent or constant tachycardia. They are emotional and generally ‘nervous.’

“I believe that when the thyroid is removed by operation for Graves’ disease and an acute thyroidism follows it can be checked by serum, but have not yet had the opportunity of thus testing it.”

The use of a specific serum in a number of other diseases has been attempted but the results up to the present time have been inconclusive or unsatisfactory.

THE NEURONS*†

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I. INTRODUCTION.

I HAVE been invited to speak on the subject of "The Neurons" and to outline to you the present state of knowledge concerning them. No topic has led to more animated discussion, perhaps, during the past ten years. Indeed, the word "neuron,"† which sprang so immediately into vogue after its introduction by Waldeyer in 1891, appears to have excited, by virtue of its rapid and widespread popularity, a feeling of bitter hostility, of unrelenting antagonism in certain circles. Hailed at its advent as the simplifier and revolutionist of our knowledge of the nervous system, and enthusiastically adopted by teachers of anatomy, physiology and pathology, the neuron doctrine has in subsequent years been subjected to the fiercest of assaults; not only has the truth of its tenets been questioned—its adversaries even go so far as to designate it as "a real danger to science."

The neurohistologic world appears to be divided into two camps, that of the neuronists and that of the antineuronists. The controversy, curiously enough, has been a rather one-sided affair, though by no means wholly so. The so-called "neuronists" have spoken and written of the nervous system as a mass of nerve units or neurons and have devoted themselves chiefly to observations and experiments concerning the internal and external morphology of these units, their relations to one

* Lecture delivered January 27, 1906.

† The author prefers the spelling "neurone" for the reasons given in his article "Concerning Neurological Nomenclature," Johns Hopkins Hosp. Bull., 1896, Nov.-Dec.

another, and especially their arrangements in chains and groups, and the relations of such chains and groups to function in health and in disease. With few exceptions they have refrained from controversy on the hypothetical side. The so-called anti-neuronists, on the other hand, while they have made some admirable contributions to real knowledge, have devoted a remarkable amount of time and energy to polemical writing; one of them is the author of a book of nearly 500 pages in which few new facts, if any, are brought forward, the whole volume being given over to a condemnation of the neuron doctrine and a denunciatory arraignment of its supporters. In the writings of the antineuronists such statements as "the neuron theory must be abandoned," "the neuron theory can no longer be held," "the neuron doctrine is false through and through," "ueber der Neurontheorie der Stab gebrochen ist" are reiterated monotonously. That the argument has become very heated at times is shown by some sample statements which I select at random from the writings of the controversialists. One of them refers to the neuronists as the "Golgi band"; another antineuronist belittles the work of a great investigator who denies networks of extracellular neurofibrils between the neurons in leeches by stating that his results are due "wholly, certainly, to the incompleteness of his preparations"; to this a neuronist has replied that "such an expression is not only very conceited, but also very partisan and juvenile." Another strong opponent of the neuron doctrine speaks of the period since when "the ominous neuron theory has animated only those who are biased." Still another remarks that "no one with normal vision can escape the convincing impression of Apáthy's specimens, provided his eye is not dimmed by envy or injured vanity." And, finally, though I could weary you with examples, Nissl states that Ramón y Cajal "is not competent to pass judgment on histologic questions," an opinion perhaps influenced by Ramón y Cajal's criticism of Nissl's "nervous gray," which he declares to be nothing but a "very cheap anatomic-physiologic conjecture, which contradicts everything that the most convincing methods teach us of detail."

From controversial statements such as these only one thing is clear, namely, that those who have made them are quarreling about matters of opinion more than about matters of fact. There is no reason why bitter dispute should long continue over easily verifiable fact. Experience teaches, however, that it is those opinions which are most feebly founded in fact and are least capable of proof that mankind is prone most passionately to defend. Science ought to try to avoid controversy by emphasizing facts and by properly estimating hypotheses. Further, scientific men should know that, once involved in discussion, nothing is gained by the bandying about of personalities; on the contrary, confidence is inspired by simple honesty in work, fairness to adversaries, moderation in statement and dignity in utterance. That was a homely but sensible saying of Truthful James when he declared that:

“I hold it is not decent for a scientific gent
To say another is an ass—at least, to all intent;
Nor should the individual who happens to be meant
Reply by heaving rocks at him, to any great extent.”

As Mr. Brander Matthews says, in his recent admirable essay entitled “Persuasion and Controversy,” in reference to convincing the public, “It is not really argument which is effective; it is information.” Give the medical profession a plain statement of fact, even in a technical field, and it will decide for itself. Not that discussion is unnecessary; quite the contrary. Criticism is essential to progress. As Schiller said to the thinkers and workers of his day, “Let there be strife among you, and the union will come quickly.”

In 1899, in my book on the “Nervous System and Its Constituent Neurons,” I tried to give an unprejudiced account of the state of knowledge at the time regarding the finer structure of the nervous system. Since then, despite the polemical activity which has prevailed, I have refrained from writing or speaking on the subject. Now that new methods have been devised and some important new facts have been discovered, the time has come when I welcome the opportunity of reviewing once more, very briefly, the actual advances which have been

made. I shall attempt, then, to-night, to pierce the dense cloud of controversial smoke which overhangs the whole neuron area in order to make as clearly visible as possible the little tongues of flame of real research hidden there. Two of these tongues are leaping high, and many think that they are likely speedily to convert the smouldering smudge of conflicting hypotheses into a brilliant blaze of fact. Ramón y Cajal lighted one of them in 1903; Ross Granville Harrison lighted the other in 1904. We shall examine them both along with other illuminating influences a little later.

II. ORIGIN OF THE NEURON CONCEPT: FACT AND THEORY IN 1891.

You are doubtless so familiar with the origin of the neuron concept that mere mention of the main factors will recall the whole story to your minds. It will be remembered that, after the nerve fibers and the so-called nerve cells were discovered, there was long great doubt as to the exact relations of these histologic elements to one another. Wagner, in 1851, and Deiters, in 1864, had made it probable that of the many processes of a multipolar ganglion cell in the anterior horns of the spinal cord only one was directly related to a nerve fiber. In 1871 Gerlach had shown that the fibers of the posterior roots do not represent processes of nerve cells of the posterior horns, but that on entering the posterior horns they divide and subdivide until they exhaust themselves and are lost in the gray matter. The question of the relation of the sensory fibers to the motor cells of the anterior horns, so important in connection with the reflexes, remained, therefore, the subject of conjecture; Gerlach advanced the hypothesis that a continuous nerve network exists throughout the gray matter of the whole central nervous system, and that all nerve cells and all nerve fibers are thus connected with one another, a hypothesis which met with almost universal acceptance among anatomists and physiologists for the next fifteen years.

In 1886 the distinguished embryologist, Wilhelm His, began to publish his careful researches on the embryology of the

nervous system. He stated in his papers that every nerve fiber is a process of an embryonic nerve cell (neuroblast); that, besides the process of the neuroblast, which becomes later the axis cylinder of a nerve fiber, other so-called protoplasmic processes (dendrites) grow out; that the single nerve cells wander for considerable distances during their development from the site of their origin; that the posterior roots of the spinal nerves are the axis cylinder processes of the spinal ganglion cells which grow into the cord, and that the anterior roots of the spinal nerves are outgrowths of axones of anterior horn cells, these outgrowths becoming subsequently covered by extracentral neurilemma cells. Most important was his idea that every nerve cell with its axis cylinder process and protoplasmic processes is a single cell, separate at the beginning from all other cells and from any end organ; connections with other nerve cells or with any end organ, like muscle or gland, His declared, either do not exist at all or, if they exist, arise secondarily. Embryology and embryologists were not so highly valued in 1886 by the rank and file of anatomists and physiologists as they are to-day and His's remarkable studies made but little impression on the strongly held hypothesis of Gerlach. As a result of pathologic anatomic considerations, especially those bearing on experimental degenerations and the resulting sharply circumscribed areas of degeneration, Forel, in 1887, came to conclusions similar to those of His and opposed to those of Gerlach and the dominant school of the day.

It was not, however, until 1888, when Ramón y Cajal began to make his striking publications of results obtained by the then little-known method of Golgi applied to the study of the embryonic nervous system, that the histologic world became convinced of the value of the ideas of His and Forel. Stroke after stroke of confirmation with the method of Golgi, which put a delicate black crust of silver over the outside of the nerve cell and each one of its processes, no matter how delicate, followed in different parts of the world. Von Kölliker, von Lenhossek and Edinger in Germany, van Gehuchten in Belgium, Retzius in Sweden, Schäfer in England, Berkeley, Starr, Strong and others

in America, repeated and extended the researches of Ramón y Cajal. Though the method is applicable satisfactorily only to embryonic tissues, its results were confirmed, in the main, especially by Dogiel and Retzius, in the tissues of the adult by the method of Ehrlich.

By the use of these two methods a newly recognized anatomic unit stood out, clearly visible in the tissues. To call it nerve cell was a little confusing, as that term had already been applied to the cell body in the gray matter independent of the related axis cylinder process. This unit included not only the old nerve cell with its dendrites, but also its axis cylinder process and all the collateral and terminal ramifications of the latter. Waldeyer¹ suggested that it be called neuron, a name which speedily found its way into all languages, including our own. Waldeyer's article, an admirable collective review of the researches in histology, embryology and pathology, undoubtedly had great influence in quickly popularizing the neuron conception. A definite anatomic fact had been established, viz., the possibility of demonstrating in embryonic tissues by Golgi's method, and in adult tissues by Ehrlich's method, a hitherto non-demonstrable unit in anatomic structure. On the basis of embryologic and pathologic work which was brought into relation with this fact, a body of doctrines, often called the neuron doctrine, was built up. A sharp distinction between the *fact* and the associated *doctrine* should be borne in mind, for much of the subsequent dispute has been due to neglect in this regard.

The associated neuron doctrine included a number of statements which at the time could be regarded as possibilities only, not as established facts. As it was formulated, it may be summarized somewhat as follows: A diffuse nerve network, in the sense of Gerlach, does not exist. There is no nerve fiber independent of a nerve cell; every nerve fiber no matter where situated, is to be regarded as the process of a nerve cell. A

¹ Waldeyer, W.: "Ueber einige neuere Forschungen im Gebiete der Anatomie des Centralnervensystems." Deutsche med. Wehnschr. Leipzig, 1891, xvii, 1244, 1267, 1287, 1331, 1352.

nerve cell with all its prolongations (dendrites and axon) constitutes a nerve unit or neuron. These nerve units are independent of one another. They are related to one another histologically and physiologically, not by continuity, but by contact; they are not united with one another anatomically or genetically. The whole of the nervous system, exclusive of blood vessels, glia and ependyma, consists of nerve units or neurons superimposed on one another. The neurons are so arranged that a nerve impulse in a given neuron always follows in one direction; it passes from the dendrites to the cell body, from the cell body to the axis cylinder process and thence to the dendrites of another neuron. All parts of a neuron are dependent on the nutritive influence of the nucleus of the cell body. The whole neuron, axon, as well as cell body, is derived from a single body cell. When a nerve fiber degenerates as a result of severance of connection with the cell body, regeneration of the axis cylinder takes place by outgrowth from the central end. See the mass of doctrines and hypotheses clinging by their tendrils to the supporting pole of anatomic fact! Here we have not only the neuron which everybody can see who looks for it, but several neuron theories which were still more or less speculative, including (1) the Cellular or Neuroblast Theory, (2) the Theory of Regeneration of the Peripheral Axon Solely by Outgrowth from the Central End, (3) the Contact Theory, (4) the Theory Denying Extraneuronal Nervous Structures, and (5) the Theory of Dynamic Polarity of the Neurons.

III. THE DISCOVERY OF THE NEUROFIBRILS: FACT AND THEORY IN

1899.

I choose 1899 as the next date of summary, for in the first place it was between 1891 and 1899 that the studies of Apáthy, Held and Bethe began to attract widespread attention and the theories of Nissl to excite comment; moreover, at the end of that year I summarized in a special volume the facts and theories bearing on the neuron up to the date of its publication.

The Hungarian histologist, S. Apáthy,² by a difficult method depending on the use of a special variety of gold chlorid, succeeded in demonstrating in the ganglion cells of various invertebrates, especially the leech, remarkable appearances of fine lines, and, in places, of networks formed by anastomosis of these lines with one another. This discovery of Apáthy is an admirable advance; it has since been manifoldly confirmed and will always stand to his credit. Confronted by such a remarkable histologic picture as the fibrils presented, he immediately began to speculate about them and advanced the following hypotheses:

(1) The neurofibrils in the nerve fibers and in the nerve cells are the especial conducting element of the nervous system.

(2) There are two kinds of cells in the nervous system—"nerve cells" and "ganglion cells"; the function of the former is to build neurofibrils which grow into and through the latter, a single neurofibril passing continuously through a series of ganglion cells.

(3) In the so-called "point substance" of Leydig, in invertebrates, neurofibrils leave the fibers and nerve cells and give rise to free extracellular networks of neurofibrils (Fig. 1).

Subsequent researches have not supported these hypotheses; indeed, the weight of recent research tends to discredit them all.

The studies of H. Held³ of Leipzig took a somewhat different direction. Golgi's method had revealed around the cell bodies of neurons (1) a pericellular network which Golgi himself believed to consist of neurokeratin and (2) a pericellular plexus of the fine terminal fibers of the axons and collaterals of other neurons. Some have thought that this plexus of fine terminal fibers formed an anastomosing network—a real "pericellular net"; others have declared that the individual fibers interlaced, but did not anastomose. Held studied these pericellular

² Apáthy, S.: "Das leitende Element des Nervensystems und seine topographischen Beziehungen zu den Zellen." *Mittheil. a. d. zool. Station zu Neapel*, 1897, xii, 495-748.

³ Held, H.: "Beiträge zur Structur der Nervenzellen und ihrer Fortsätze," *Arch. f. Anat. u. Entwicklungsgesch.*, Leipzig, 1897, 204-294; also, supplement volume, 1897, 273-312.

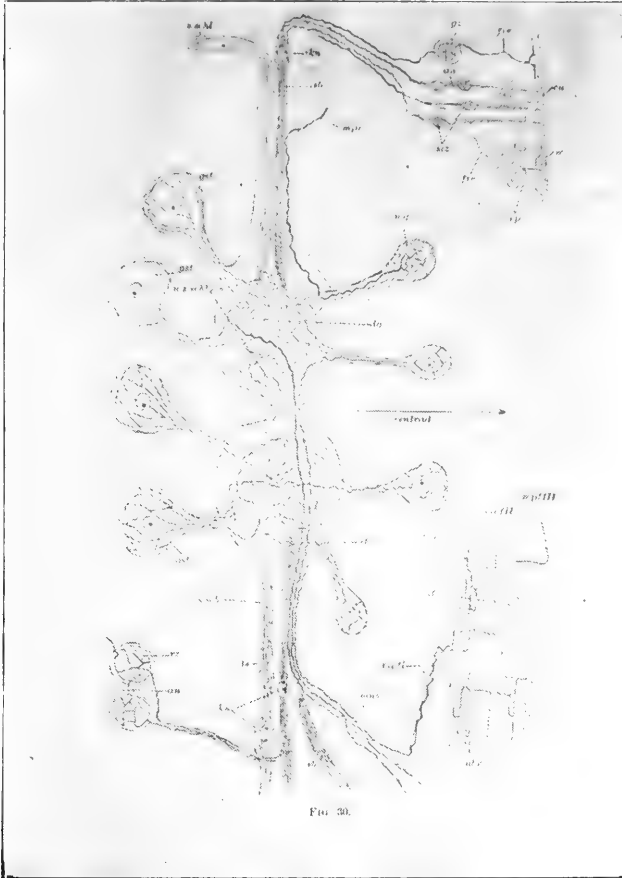


FIG. 30.

FIG. 1.—Apáthy's schematic representation of the course and connections of the conducting paths in a transverse section of the somite of the leech. (After S. Apáthy, *Mitth. a. d. zool. Station zu Neapel*, 1897, vol. xii, plate 2, Fig. 6.)

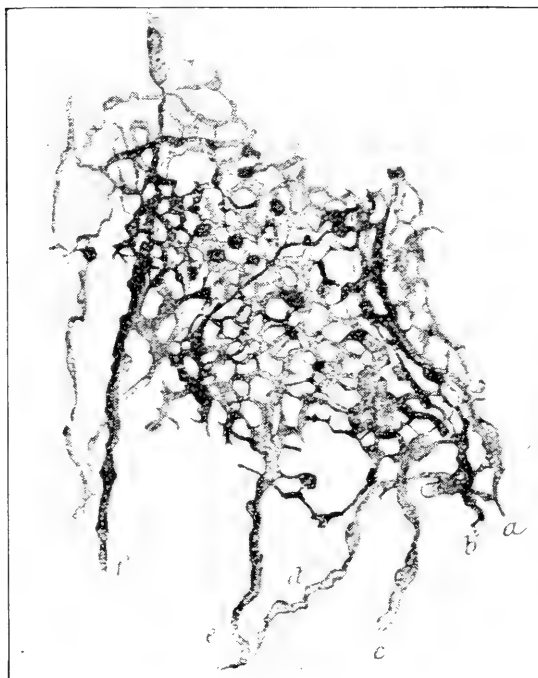


FIG. 2.—Part of the network around a cell in Deiter's nucleus. The paler network is the Golgi net. Held believed that the thickenings in the network correspond to his "end-feet." He believed that the axons helped to form this network. Recent investigations do not corroborate this view. The Golgi net is now believed by Held himself to be glia. (After H. Held, 1897.)

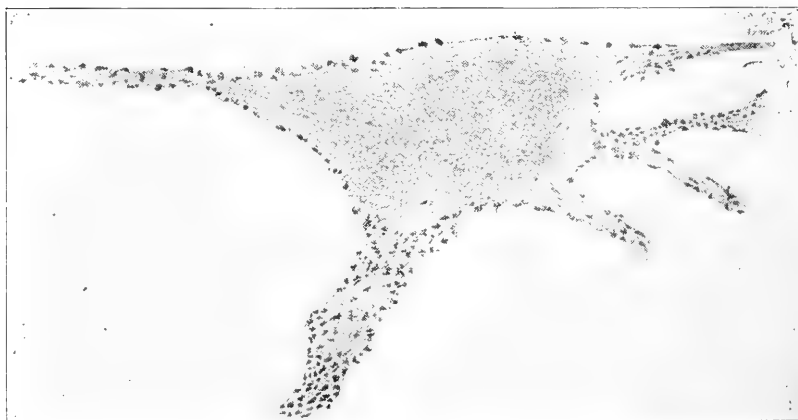


FIG. 3.—Cell from the ventral horn of the lumbar cord of an adult rabbit, showing masses of neurosomes in the "end-feet" terminating on the dendrites and cell body. (After H. Held, 1897.)



FIG. 4.—Ganglion cells with fibrils stained by Bethe's method. *A*, human anterior horn cell of man ; *B*, cell from nucleus of facial nerve of rabbit, Nissl's bodies also shown ; *C*, dendrite of a human anterior horn cell ; *D*, two human pyramidal cells. (After Bethe, 1900.) Bethe's method showed isolated fibrils, but was not delicate enough to show the anastomoses among the fibrils.



FIG. 5.—Nissl's scheme of the "nervous gray." The left half of the scheme shows the facts as known according to Nissl. In the right half Nissl's hypothesis of the structure of the nervous gray is illustrated.

structures by various histologic methods and found that the terminals of nerve fibers end in delicate expansions—so-called “end-feet” (*Endfüsse*)—recognizable by the large number of minute neurosomes which they contain on the surface of the cell body and its dendrites, fusing, he believed, by actual conescence, with the protoplasm. He saw, he believed, delicate connections of these “end-feet” with one another in the form of an anastomosing network, so that he advanced the idea of two kinds of interneuronal connection, (1) anastomosis of the terminals of axons derived from different neurons in a pericellular network (Fig. 2) and (2) direct continuity of the protoplasm of the end-feet with the protoplasm of the cell to which they are attached (Fig. 3).

Next in order came the researches of Bethe.⁴ Everyone who had tried Apáthy's method of staining neurofibrils had failed with it, and when Bethe announced his discovery of an easier method of demonstrating them it was gladly welcomed. The method had the advantage, further, of staining the neurofibrils in vertebrates, including man. His method showed, he asserted, independent, non-anastomosing neurofibrils running through the cell body or passing from one dendrite to another without passing through the body of the cell (Fig. 4). He regarded the neurofibrils as the sole conducting element in the nervous system. His method showed also a pericellular network which he called the “Golgi net.” It seemed to Bethe probable that this Golgi net was the medium of communication between the neurofibrils of the nerve fibers terminating about the cell and the neurofibrils inside the cell; according to this hypothesis the neurofibrils would represent a continuous conduction system throughout the whole nervous system, the so-called neurons representing, as it were, a bed in which the essential neurofibrils lie.

⁴ Bethe, A.; “Ueber die Primitivfibrillen in den Ganglienzellen vom Menschen und andern Wirbelthieren,” *Morphol. Arb.*, 1897, viii, 95–116; also “Ueber die Neurofibrillen in den Ganglienzellen von Wirbelthieren und ihre Beziehungen zu den Golginetzen,” *Arch. f. mikr. Anat.*, Bonn, 1900, lv, 513–558.

In 1898 F. Nissl⁵ launched his hypothesis (more fully developed in 1903) of the structure of the gray matter. He called attention to how little is really known about the gray matter, information yielded by Golgi's method not being regarded as of any value. Nerve fibers we know and nerve cells we know, but in the gray matter there must be something specific which is a mystery. This mystery he designates *nervöse Grau*, or "nervous gray." He lays great stress on the Golgi nets around the nerve cells; he asserts that histologic methods leave us in the lurch when we seek to follow axis cylinders into the gray matter beyond the point where the myelin sheaths cease. There is thus an area around nerve cells and between them and the medullated nerve fibers which is filled up with "nervous gray," for him the most important constituent of the whole nervous system. While this is all we really know, Nissl says, still we must suppose that this nervous gray is so constructed that it conducts nerve impulses and permits the reciprocal action of the elements of the nervous tissues on one another. He has devised a most extraordinary hypothesis to account for this function of the "nervous gray." Like Bethe's theory, it involves the communication of the neurofibrils in the nerve cells by way of the Golgi nets with a great complex of neurofibrils outside the Golgi nets (Fig. 5).

Thus far knowledge and theory had advanced by 1899, and in my book, and in an article entitled "The Validity of the Neuron Doctrine," published in that year, I called attention to these phases of the subject, advised active skepticism as regards the new hypotheses of Apáthy, Bethe and Nissl, and expressed the opinion that, even if the neurofibrils really represent a constant constituent of the nerve elements, and even if the inter-neuronal relation be a more intimate one than that of mere contact or contiguity, the essential feature of the neuron conception remains unaltered, Waldeyer having stated in an article in which he gave the neuron its name that "if we assume

⁵ Nissl, F.: "Nervenzellen und grauen Substanz," Münch. med. Wehnschr., 1898, xlv, 988; 1023; 1060.

. . . the existence of nerve networks, the conception is somewhat modified, but we can still retain the nerve units. The limits between two nerve units would then always lie in a nerve network and not, anatomically at least, be exactly definable with our present methods." I accompanied my review, therefore, at that time, with the statement, "There may be units smaller than cells, and in all probability there are; there may be, and probably are, in the nervous system units other than those generally described, and it is important that we should find out all that there is to learn about them; but that the human body is made up largely of a mass of cells, and that the human nervous system is made up largely of great numbers of cell units, the so-called neurons, would seem to be facts too firmly established ever utterly to be overthrown."

IV. MORE RECENT WORK: FACT AND THEORY TO-DAY.

During the next five years (1899-1904) the subject of neuro histology was enriched by a mass of detailed investigative work.⁶ Many studied the neurofibrils and extended our knowledge of them, among them Paton, Prentice, and Biart in America. In addition, the period of polemical writing on the neuron and the neuron doctrines reached its zenith. Edinger, in 1898, had urged the importance of the concept of the neuron as a biologic unit; Hoche (1899) had admitted that the histologic unity of the neuron could no longer be upheld, but urged that it was a functional-trophic unit. Münzer (1899) took the stand that the neuron doctrines were better founded than ever, and that the criticisms of Nissl and Bethe were without weight. Auerbach (1899) supported a modified neuron doctrine, while Semi Meyer, von Lenhossék, van Gehuchten, and Ramón y Cajal could see no reason for changing the original conception and doctrines. Max Verworn, in a collective review read before the German Society of Natu-

⁶ See the review by Prof. G. E. Coghill, "Recent Studies on the Finer Structure of the Nerve Cell," *J. Comp. Neurol. and Psychol.*, Granville, O., 1904, xiv, 171-199.

ralists in 1900 took almost precisely the stand I took in 1899. The two longest publications of the antineuronists are those of Bethe (1903) and Nissl (1903). Bethe's book,⁷ while it represents a vigorous attack on the neuron doctrine, embodies the results of most interesting and important original researches, and should be carefully read by every neurologist. Nissl's large volume,⁸ which was reviewed for the *Psychological Bulletin* by Dr. Adolf Meyer, is devoted entirely to controversy and is looked on by ardent neuronists as a sort of attenuated curse of St. Ernulphus on the neuron doctrine and its supporters.

In December, 1903, by good fortune, I happened to be in Madrid just at the time when Ramón y Cajal⁹ announced his discovery of a new and easy method for demonstrating the neurofibrils. He kindly showed me his preparations, which made clear at a glance that a flood of new light was at once to be thrown on these remarkable constituents of the nerve cells and nerve fibers (Figs. 6, 7 and 8). His method is simple, is easily applicable to invertebrate as well as to vertebrate tissues and to the embryonic nervous system as well as to that of the adult. It stains the fibrils much more perfectly than is possible with the methods of Bethe and Apáthy, and has the further advantage that it leaves the glia entirely unstained. A somewhat similar, though more complex, method, devised independently and almost simultaneously by Bielschowsky¹⁰ of Berlin,

⁷ Bethe, A.: *Allgemeine Anatomie und Physiologie des Nervensystems*, Leipzig, 1903, 488 pp.

⁸ Nissl, F.: *Die Neuronenlehre und ihre Anhänger*, Jena, 1903, 478 pp.

⁹ Ramón y Cajal, S.: "Un sencillo método de coloración del retículo protoplasmático y sus efectos en los diversos centros nerviosos de vertebrados é invertebrados." *Trab. del Lab. de investig. biol.*, Madrid, 1903, ii, No. 4; also, *Translation by Azoulay, Bibliogr. anat.*, Paris, and Nancy, 1905, xiv, 93 pp.

¹⁰ Bielschowsky, M.: "Die Silberimprägnation der Neurofibrillen," *Neurol. Centralbl.*, Leipzig, 1903, xxii, 997-1006; also "Ein neues Imprägnationsverfahren zur Darstellung der Neurofibrillen." *Arch. f. Psychiat.*, Berlin, 1905, xxxix, 1321-1323.

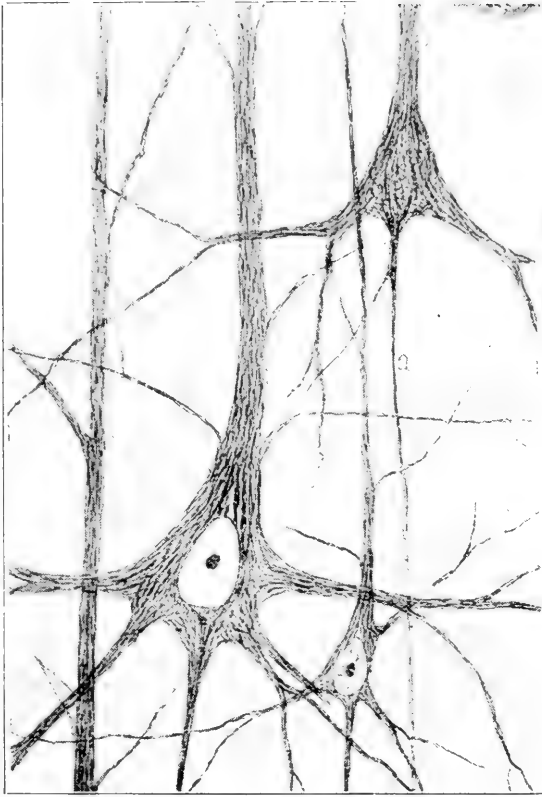


FIG. 6.—Large and medium-sized pyramidal cells from the human visual cortex.
a, Axis cylinder. (After Ramón y Cajal, 1903.)

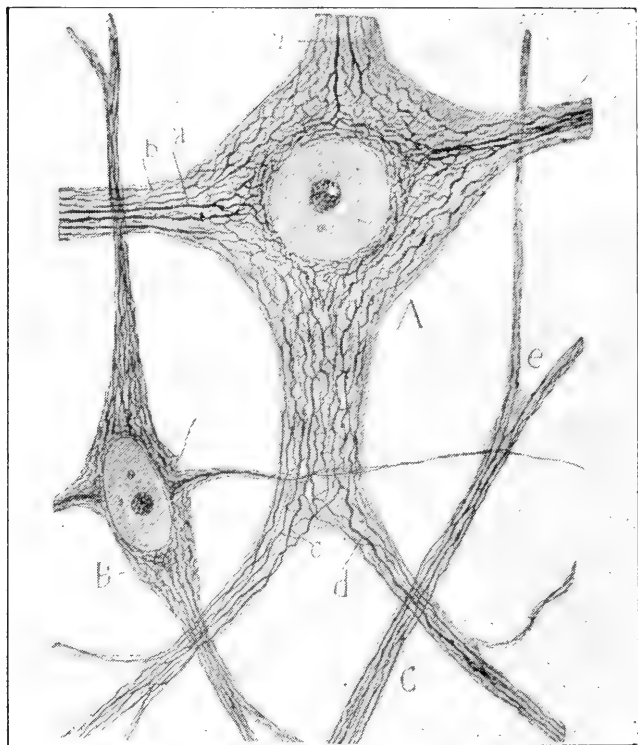


FIG. 7.—Funicular cells from a rabbit fifteen days old. *A*, large cell; *B*, small cell; *a*, large neurofibrils ramifying in the perinuclear network; *b*, fine neurofibrils continuous with the cortical network; *d*, *e*, neurofibrils bifurcating on their arrival at a large dendritic trunk. (After Ramón y Cajal, 1903.)

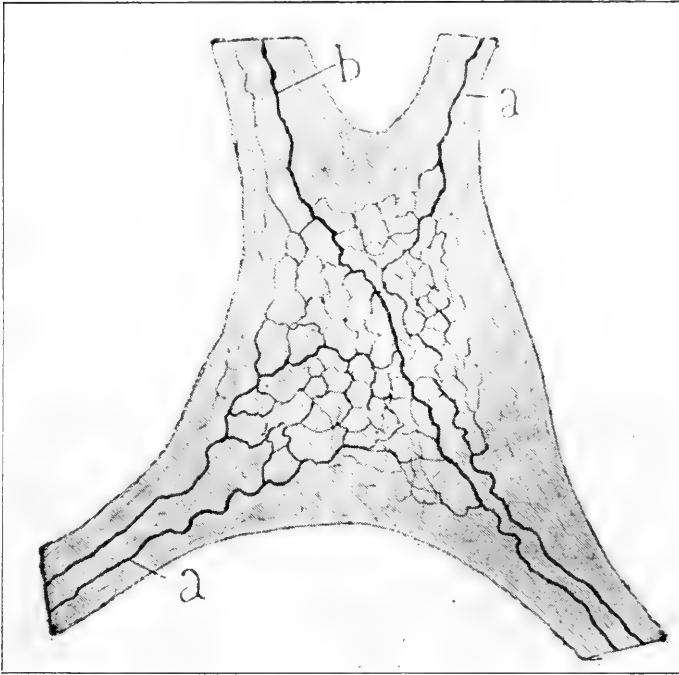


FIG. 8.—Large funicular cell from a rabbit eight days old. Certain only of the primary neurofibrils have been drawn. The network formed by the anastomoses of these filaments is well shown. *a*, neurofibrils terminating in the network; *b*, primary neurofibril running through. (After Ramón y Cajal, 1903.)

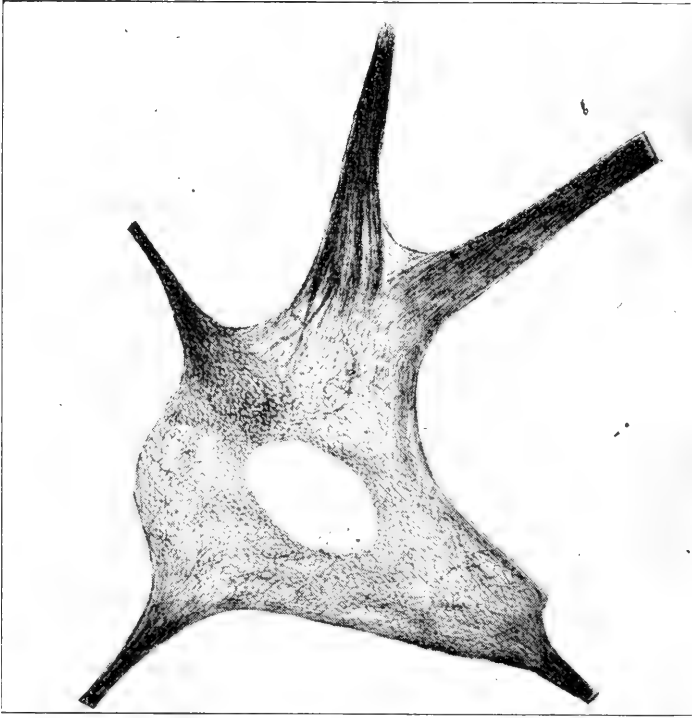


FIG. 9.—Cell from the spinal cord. (After Donaggio.)

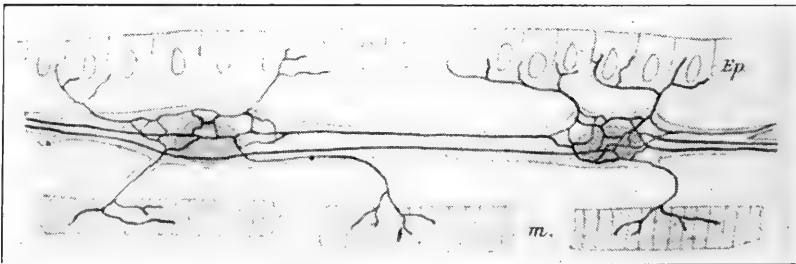


FIG. 10.—Scheme of the course of the neurofibrils in a nerve network in lower animals (After Bethe, 1903.) Not confirmed by recent researches.

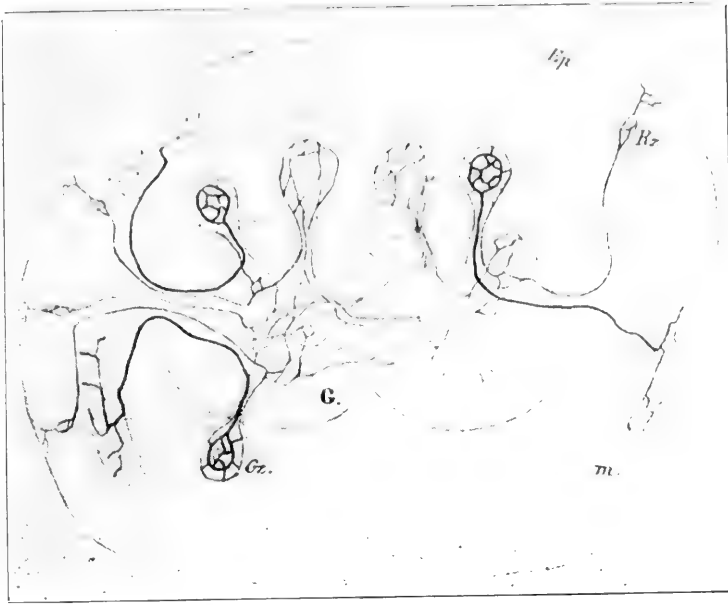


FIG. 11.—Scheme of the course of the neurofibrils in the nervous systems of worms. *G*, ganglion; *Gz*, ganglion cells; *Rz*, reception cells. (After Bethe, 1903.) Not confirmed by recent researches.

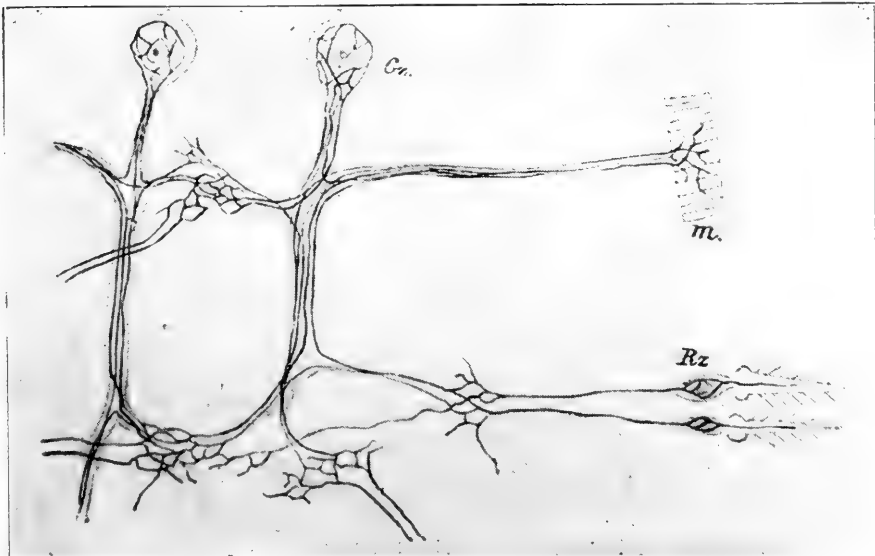


FIG. 12.—Scheme of the course of the neurofibrils in the nervous system of the crab. (After Bethe, 1903.) Not confirmed.

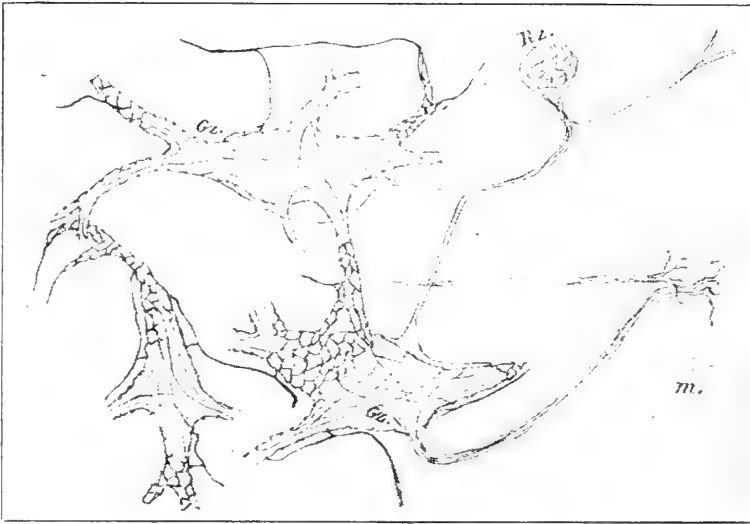


FIG. 13.—Scheme of the course of the neurofibrils in the nervous system of vertebrates. (After Bethe, 1903.) Relation to the Golgi net is shown. Not confirmed by newer work.

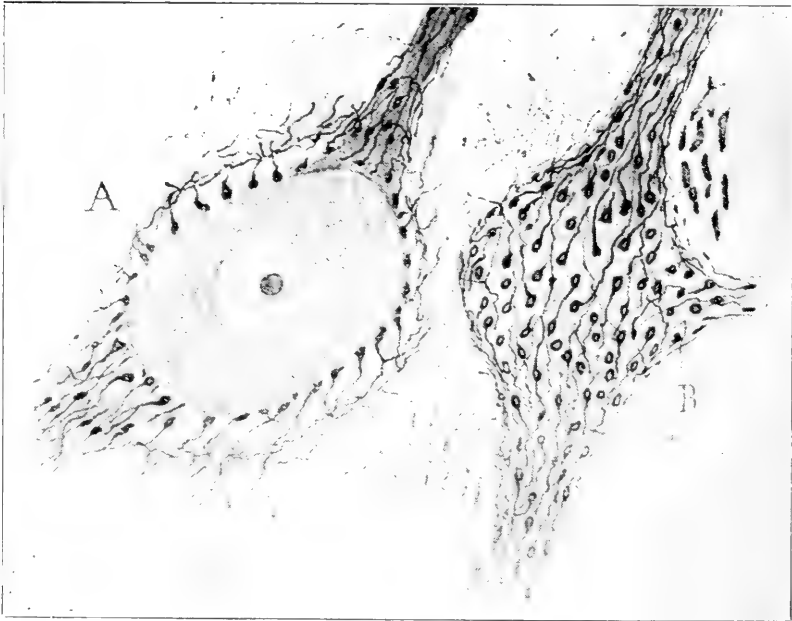


FIG. 14.—Two large funicular cells of the spinal cord of the adult rabbit. *A*, section through a cell showing terminal buttons of Auerbach ending on the surface of the cell and on the dendrites; *B*, terminal buttons shown on the surface of the cell. (After Ramón y Cajal, 1903.)



FIG. 15.—Transverse cut through a ganglion of the leech showing the neurofibril networks in the ganglion cells and the non-anastomosing plexus of neurofibrils in the point substance. (After Ramón y Cajal, 1903.)

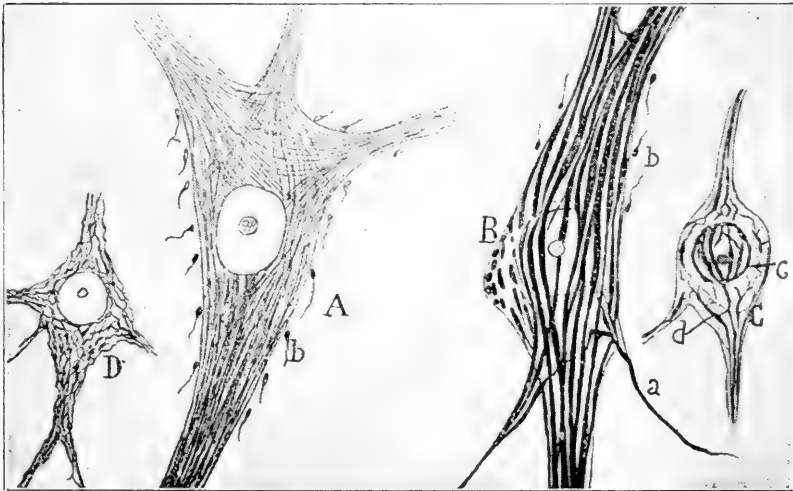


FIG. 16.—Cells of the spinal cord of the lizard. *A, D*, motor and funicular cells in the state of activity (lizard kept for three hours in the thermostat at 30). *B, C*, motor and funicular cells in the state of repose (lizard kept at a temperature of 12). *a*, axis cylinder; *b*, terminal buttons of other axons; *c*, perinuclear network; *d*, thickened primary neurofibril. (After Ramón y Cajal, 1903.)

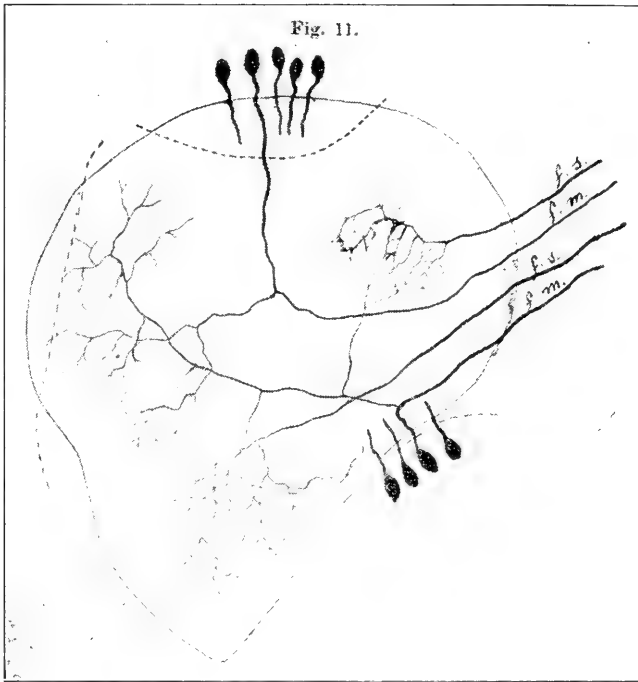


FIG. 17.—So-called “fundamental experiment” of Bethe. The drawing indicates the position of the motor cells of the neuropile in the ganglion of the crab. The cells were cut away as indicated by the dotted lines. f. s., sensory fiber, f. m., motor fiber. (After Bethe.)

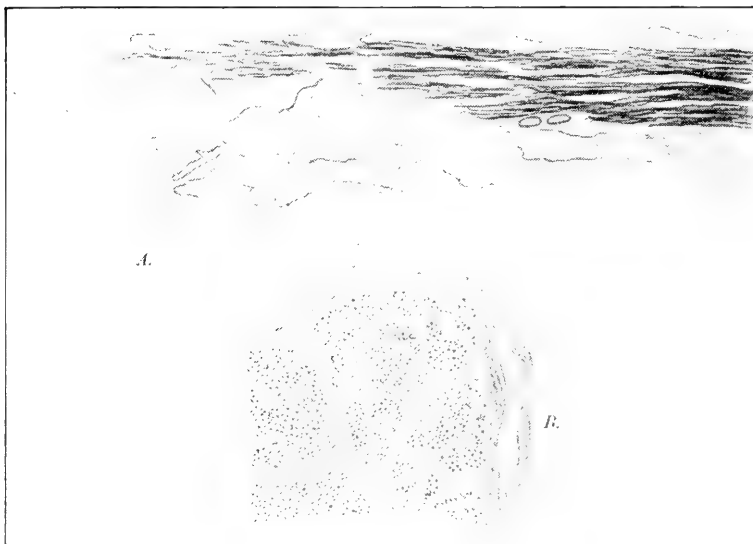


FIG. 18.—A, longitudinal section through an “autoregenerated” nerve; B, transverse section through the same nerve a little further distalward. (After Bethe, 1903.)

is also now much used for demonstrating the neurofibrils. The finest fibrils are said to be best shown by still a third method, that of the Italian investigator, Donaggio¹¹ (Fig. 9).

Let us now turn to an examination of the principal points which have been under discussion of late, and see what is the actual state of knowledge concerning them.

(a) *The Neurofibrils, Golgi Nets and Interneuronal Relations*.—Studies by the methods of Cajal and Bielschowsky and by the method of Donaggio have shown how rich and delicate the network of neurofibrils in the nerve cells is. Bethe's method stained only the coarser strands, which, therefore, looked like isolated fibrils. The newer methods demonstrate finer fibrils, forming anastomoses among these. Bethe's schemata (Figs. 10, 11, 12, and 13) of varying arrangements of neurofibrils as the animal scale is ascended are not confirmed by these newer methods. On the contrary, the nerve cells as regards the neurofibrils are similarly built throughout the whole animal series. There appear to be no independent neurofibrils, but only neurofibril networks embedded in the nerve-cell protoplasm (see figures). The network extends through the whole neuron, but the mass of the network may increase or diminish in size in various parts. Thus there is a large fibril network in the cell body, and this is connected by the attenuated fibril network of the axon, with expansions of the network in the terminal fibers. The so-called "end-feet" of Held are now known to be identical with the terminal buttons of Auerbach, and these, ending on the surface of the cell body and dendrites, are exquisitely demonstrated by Cajal's method (Fig. 14). I have placed a specimen under the microscope illustrating them. These terminal buttons contain neurofibril networks. Nowhere, in specimens stained by Cajal's method or by Donaggio's method, in the cell body or in the terminal buttons, thus far have the neurofibrils been seen actually at the surface; it

¹¹ For an epitome of Donaggio's method and work, see Donaggio, H.: "The endocellular fibrillary reticulum and its relations with the fibrils of the axis-cylinder." *Rev. Neurol. and Psychiat., Edinb.*, 1905, iii, 81—100, 3 pl.

is stated that there is always an area of fibril-free protoplasm between the networks and the cell boundary.

These recent methods which stain the neurofibrils so exquisitely do not reveal any extraneuronal neurofibrils whatever, either in the region of Nissl's "nervous gray" (Cajal, Retzius) or in the so-called point substance or neuropile of invertebrates (Cajal, Retzius) (Fig. 15). The whole area of Nissl's nervous gray appears in Cajal's specimens as a feltwork of dendrites and terminals and collaterals of axons containing neurofibrils within their protoplasm. The Golgi nets do not stain at all with Cajal's neurofibril method. Moreover, Held, by other methods, has concluded that they are derivatives of the glia; both he and Donaggio deny their nervous character. There is some evidence that the terminal buttons, to which I have referred, reach the protoplasm of the nerve-cell body by lodging in the meshes of the Golgi net. No one has been able to demonstrate any relation between neurofibrils and Golgi nets. In the light of these newer studies the enormous importance attributed by Bethe and Nissl to the Golgi nets seems, therefore, to have been premature; their views are unsupported by facts.

The next questions to be decided are, (1) Do the minute fibers ending in the terminal buttons form anastomoses with one another? and (2) Do neurofibrils pass from the terminal buttons into the adjacent cell body or dendrite to form connections with the neurofibrils lying in the protoplasm there? I am unable to find them in the preparations I have been studying, nor can Ramón y Cajal, von Lenhossék, Retzius, van Gehuchten or Mahaim in theirs. Held¹² thinks that he sees such communications, and Max Wolff is inclined to a similar view. Further study is needed to decide this point. Ramón y Cajal is so convinced of the separateness of the terminal buttons from the adjacent nerve-cell protoplasm that he unhesitatingly assures us that not only is the neuron conception valid, but even the contact doctrine is better supported now than ever

¹² Held, H.: "Zur Kenntniss einer neurofibrillären Continuität der Wirbelthiere." *Arch. f. Anat. u. Physiol.*, Leipzig, 1905, 55-76.

before, and Sherrington¹³ takes the ground that the cell membranes at the junctions of the neurons (synapses) may be of very great importance in the reflex processes.

Certain it is, if one makes a quick review of all the theories which have been advanced, with the aim of discrediting the views based on the findings with Golgi's method, one comes necessarily, as van Gehuchten has emphasized, to a double conclusion: First, none of the theories opposing the neuron conception has led to the objective demonstration of the existence of a real continuity among the nerve elements, and, second, there is a marked difference between so-called neuronists and so-called antineuronists in interpreting known facts. The former, faithful to the facts observed, declare that in their preparations they find only free ramifications, and, not being able to see intercellular anastomoses, they maintain that one should not admit that they exist. The opponents of the neuron conception affirm that intercellular anastomoses ought to exist, but, not being able to demonstrate them, they themselves supply what is lacking in their preparations; using theoretical considerations and physiologic arguments as a basis, they construct the desired continuity out of whole cloth. Advocates of the neuron conception and of the contact doctrine naturally regard this negative result of the numerous efforts made to establish the continuity as a very convincing argument in favor of the real independence of the neurons. Opponents of the neuron conception think that continuity is, *a priori*, so probable that those who deny it should bring the absolute proof that it does not exist.

May it not be possible that the multiplication of hypotheses has been largely due to the supposition, thus far baseless, that the neurofibrils represent the sole conducting element in the nervous system? In an address delivered in 1899,¹⁴ commenting on this subject, I said: "Some investigators have been

¹³ Sherrington, C. S.: "Ueber das Zusammenwirken der Rückenmarksreflexa und das Prinzip der gemeinsamen Strecke." *Ergebn. d. Physiol.*, Wiesb., 1905, iv, 797-850.

¹⁴ Barker, L. F.: "The Progress of Neurology," *Yale Medical Journal*, 1899.

tempted, very naturally, I think, to assume that the fibril-like structures in the ground substances represent the essential conducting substance, but, however plausible, this is not yet satisfactorily proven, and, even if such structures were shown to be particularly suited for such conduction, a similar function for other parts of the nerve-cell protoplasm would by no means be excluded." Bethe and Mönckeberg's argument that only neurofibrils pass through the nodes of Ranvier has no weight if it be true, as is asserted, that perifibrillar and interfibrillar protoplasm also pass through the nodes. There can be but little doubt that the neurofibrils are of some special importance for the cells, but we are as yet as ignorant of the function they subserve as we are of the functions of the fibrils in the muscle cells and in other cells of the body. Schiefferdecker¹⁵ suggests that the fibrils are not for the purpose of isolated conduction, but that they, together with the plasma, produce a definite chemical transformation in the neuron, which is propagated through the axon and is able to excite other nerve cells or end organs.

In hibernating animals the neurofibrils are quite different in appearance from those in active animals (Fig. 16). Studies of the neurofibrils in pathologic conditions have already been begun (Marinesco, Bellot *et al.*), and it is found that they undergo definite changes when the neurons are injured.

(b) *The Neuron as a Physiologic Unit.*—Under this heading I shall refer to two points only: 1. The possibility of nerve conduction in the absence of the cell body of the neuron. 2. The so-called "auto-regeneration" of the peripheral nerves.

With regard to the first of these two points, Bethe's so-called fundamental experiment has attracted much attention (Fig. 17). In studying the nervous system of a crab (*Carcinus mænas*), he cuts off the motor cells at the periphery of the gan-

¹⁵ Schiefferdecker, P.: "Nerven- u. Muskelfibrillen, das Neuron und der Zusammenhang der Neuronen," Deutsche med. Wchnschr., 1905, xxxi, 613; also, "Ueber die Neuronen und die innere Sekretion." Sitzber. d. Niederrhein. Gesellsch. f. Natur d. Heilk., Bonn, 1905, Oct. 23.

glion of the second antenna, and finds that as long as the sensory fibers (F. s.) and motor fibers (F. m.) of the antenna remained intact and in connection with the cell-free neuropile of the center of the ganglion the antenna behaved normally; the tonus of the muscles is preserved and the reflexes persist unaltered. Bethe thinks this experiment proves conclusively that the cell body, the part of the neuron containing the nucleus, is not necessary for the reflex functions, and that the normal tonus of the muscles does not depend on the cell body, excitation of the sensory fibers being transmissible to the centrifugal fibers in spite of the entire absence of nerve cells. If this experiment be confirmed, it will show that the whole neuron does not necessarily enter into every function, or in other words, that there may be nerve functions which can be carried out with utilization of a part only of the cell. The experiment points also to the validity of the hypothesis, advanced by Ramón y Cajal and van Gehuchten, of the axipetal conduction function of the dendrites. It was thought at first to demonstrate the conducting function of free extracellular neurofibrils, but recent studies indicate, as has been already said, that the neurofibrils of the neuropile are all embedded in the protoplasm of the processes of nerve cells.

The question of the possibility of auto-regeneration of the distal end of a divided nerve which has been prevented from uniting with its central end is one of very great interest. Bethe has repeated the earlier experiments of Philippeaux and Vulpian, and asserts that in young animals auto-regeneration takes place, the Schwann cells, uniting end to end, building the new nerve fibers and producing not only new axons, but actually new myelin sheaths and neurofibrils (Fig. 18). Bethe's experiments have been confirmed by Ballance and Stewart in England, van Gehuchten in Belgium, Barfurth¹⁶ in Germany, and recently also by Raimann.¹⁷ The validity of the experiments

¹⁶ Barfurth, D.: "Die Regeneration peripherer Nerven." *Anat. Anz.*, Jena, 1905, xxvii, supplement, 160-172.

¹⁷ Compare abstract, *Neurol. Centralbl.*, Leipzig, 1905, 1015.

has been denied by Munzer and by Langley and Anderson,¹⁸ the latter asserting that if anastomosis with other nerves in the limb and all possibility of outgrowth from the central stump be prevented no auto-regeneration occurs. The supporters of auto-regeneration are positive that they prevent any connection of the central stump of the sciatic, the nerve experimented on, with the distal stump of the divided nerve, Raimann having even excised the portion of the spinal cord and spinal ganglia corresponding to the origin of the sciatic nerve in order to prevent absolutely any central outgrowth. In view of Frossmann's¹⁹ interesting experiments, which show the powerful positive attractive force (neurotropism) exerted by disintegrating nerve substance on living nerve fibers at a distance, it has become necessary to think not only of an outgrowth from the central stump of the *N. ischiadicus*, but also from branches of the other nerves of the lower extremity, namely, the *N. femoralis* and *N. obturatorius*. To test this point Lugaro of Florence²⁰ excised the whole lumbosacral cord and the corresponding spinal ganglia in three puppies. After three months the nerves of the limbs were found to be faradically totally inexcitable, and histologic examination showed entire absence of any autogenous regeneration. It is highly important that a series of similar experiments be performed, and that the truth in this matter be finally determined. Should auto-regeneration actually occur, we should have another wonderful example of the power of adaptation of the body in utilizing in regeneration histologic elements of entirely different embryonic origin from those by which the structure is first formed. It would not be any more wonderful, however, than the generally accepted regeneration of an extirpated crystalline lens by the iris epithelium in Triton.

¹⁸ Langley and Anderson: "Autogenic Regeneration in the Nerves of the Limbs." *J. Physiol., Lond. and Camb.*, xxxi, 1904.

¹⁹ Frossmann: "Ueber die Ursachen welche die Wachstumsrichtung der peripheren Nervenfasern bei der Regeneration bestimmen." *Beitr. z. Path. u. path. Anat., Jena*, 1898, xxiv; also "Zur Kenntniss des Neurotropismus," *ibid.*, 1900, xxvii.

²⁰ Lugaro, E.: "Zur Frage der autogenen Regeneration der Nervenfasern." *Neurol. Centralbl., Leipzig*, 1905, 1143-1144.

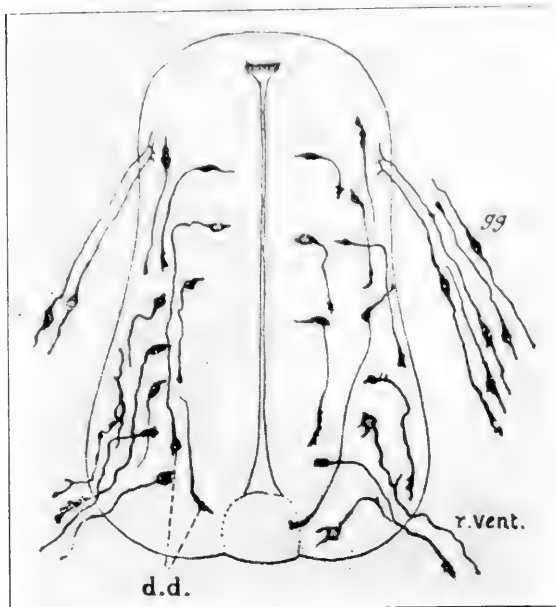


FIG. 19.—Section of spinal cord of a chick at the third day of incubation. (After Ramón y Cajal.) *g g*, cells of spinal ganglion; *d d*, ends of cells on which the dendrites develop later. At the opposite poles are shown the embryonic axons, at the extremities of some of which there are bulbous swellings. *r. vent.*, ventral root.



FIG. 20.—Transverse section through an embryo of chick after an incubation of two days, twenty-one hours. The bands of spindle-shaped cells in the course of the spinal nerves illustrated. (After Bethe, 1903.)

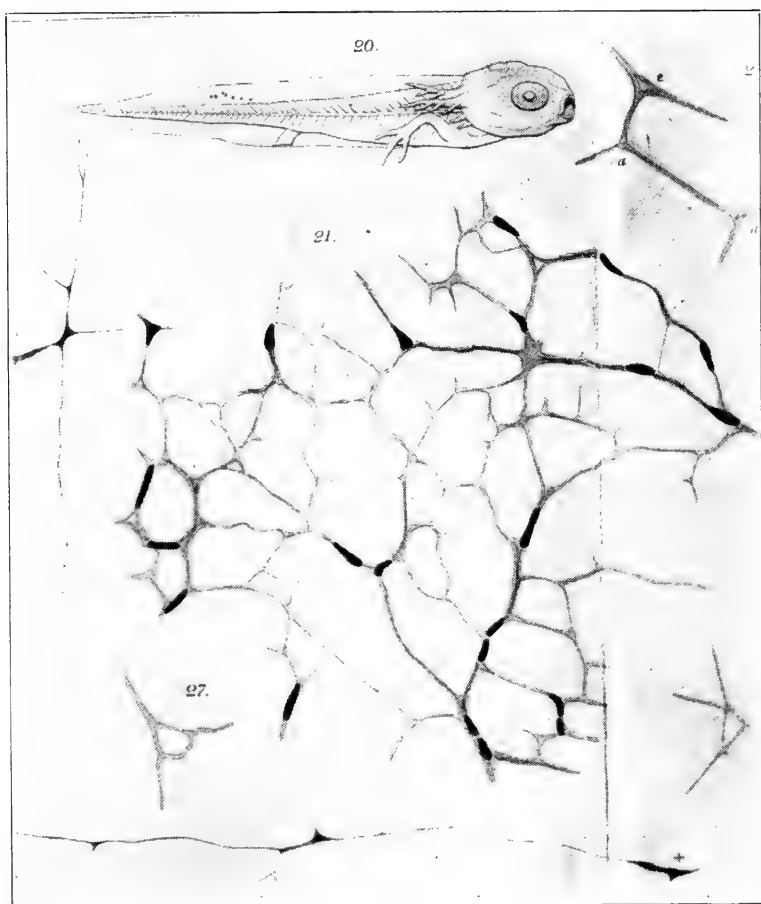


FIG. 21.—Peripheral nerve network. (After O. Schultze.)

(c) *The Neuron as an Embryologic or Cellular Unit.*—The embryologic researches of His, which taught that the axis cylinder of a nerve fiber represents the outgrowth from a single nerve cell, had, as we have seen, much to do with the origin of the neuron conception. The neurilemma cells forming the sheath of the peripheral nerve fibers were regarded as accessories for protective or nutritive purposes. The studies of Golgi's preparations of young embryos confirmed in the most striking way the opinions of His (Fig. 19).

This doctrine of the unicellular origin of the neuron has by no means gone unchallenged. Indeed, there is a large school of investigators to-day who maintain that the peripheral nerve fibers, inclusive of their axons, arise in the embryo as the result of the fusion of long chains of cells placed end to end. This pluricellular or catenary explanation of the origin of the peripheral nerve fibers has been extended even to the dendrites and the nerve cell of the central organs, certain Italian investigators especially asserting that the rows of cells fuse inside the central system to give rise to them, their nuclei gradually disappearing.²¹

Bethe recently undertook again the study of the development of the peripheral nerve fibers from the embryologic side, and published his results in his book of 1903. He states that, before the appearance of any trace of peripheral nerve fibers, a band of spindle-shaped cells can be seen in the place where the nerve is to be formed, and it is these cells, he believes, which produce by differentiation of their protoplasm the neurofibrils of the peripheral nerve fibers and the nuclei of their internodal segments (Fig. 20). Bethe looks on this cellular band as a true syncytium, the protoplasmic part of which builds filaments, which, extending from cell to cell, finally all fuse together and

²¹ Compare Capobianco and Fragnito: "Nuove ricerche su la genesi ed i rapporti mutui degli elementi nervosi e neuroglici." *Ann. di nevrol.*, 1899, xvii; Pighini, G.: "Sur l'origine et la formation des cellules nerveuses chez les embryons de Sélaciens," *Bibliogr. Anat.*, Paris and Nancy, xiv, 74-105; La Pegna, E.: "Su la genesi ed i rapporti reciproci degli elementi nervosi nel midollo spinale di pollo," *Ann. di nevrol.*, 1904, xxii.

become continuous with the nerve cells in the centers. Gradually each of these filaments becomes surrounded by nuclei and myelin sheath, and a large number of individual nerve fibers ultimately arise from the cellular band. Similar views of the origin of the peripheral fibers have been advanced by Apáthy and Sedgwick.

Hensen²² has for forty years opposed the doctrine of an outgrowth of free nerve endings from the centers to the end organs. It is his opinion that, from the earliest stages of development, nerve-cell and end organ are connected by long-drawn-out intercellular bridges. As the cells go on dividing, the connecting intercellular bridges also divide more or less completely. The divided nerves gradually become separated from one another as growth proceeds. Since many of the subdivisions are incomplete, there finally arises an interminable network of fibers. Hensen then assumes that certain portions of this network become useful to the body as a nervous system and persist; the unused portion of the network atrophies and disappears. The peripheral networks have been carefully studied of late by an especial technic by O. Schultze.²³

Up to 1904 it had become ever clearer that unanimity of interpretation of the histologic pictures of developing nerves was not to be arrived at unless some new and convincing method could be devised which would settle the question definitely. In April of that year Braus²⁴ published the results of some very interest-

²² Hensen, V.: "Ueber die Entwicklung des Gewebes und des Nerven im Schwanze der Froschlarve." *Virch. Arch.*, 1864, xxxi, 51; "Ueber die Nerven im Schwanze der Froschlarven." *Arch. f. mikr. Anat.*, Bonn., 1868, iv, 111-124; *Die Entwicklungsmechanik der Nervenbahnen im Embryo der Säugetiere*, Kiel and Leipzig, 1903.

²³ Schultze, O.: "Beiträge zur Histogenese des Nervensystems. I. Ueber die multizelluläre Entstehung der peripherensensiblen Nervenfasern und das Vorhandensein eines allgemeinen Endnetzes sensibler Neuroblasten bei Amphibienlarven." *Arch. f. mikr. Anat.*, Bonn, 1905, lxvi, 41-110, 4 pl.

²⁴ Braus, H.: "Experimentelle Beiträge zur Frage nach der Entwicklung peripherer Nerven." *Anat. Anz.*, Jena, 1905, xxvi, 433-479.

ing experiments on tadpoles (Bombinator). At the period of appearance of the fore limb as a minute bud, he excised this bud and transplanted it to a point between the bud for the hind limb and the root of the tail. The bud grew and gave rise to an extremity quite like a fore limb, only out of position. At the time of transplantation the limb already contains the rudiments of nerves and blood vessels. This differentiation, according to Braus, recedes during the next few days after transplantation and the tissues of the bud again come to resemble an indifferent blastema. Braus, following the idea of Roux, believes that this bud in its further growth leads its own life, that development goes on in its tissues by autodifferentiation. Certain it is that the blood vessels, bones, muscles and nerves develop in it. As early as three weeks after the operation the nerves in the transplanted limb present a development equal to that of a normal limb. Though Braus believes that these nerves have developed "autogenetically," he admits that they are connected by means of three strands with the general nervous system of the tadpole; he feels sure, however, that these strands are too delicate to have served for the passage from the central nervous system to the limb of all the peripheral nerve fibers to be found there. It looked, therefore, at first as though the doctrine of the pluricellular origin of the peripheral nerve fibers were about to receive support from the experimental side.

In the summer of the same year (1904), however, the subject was approached in a most ingenious way by one of our American anatomists, Dr. Ross Granville Harrison, of Baltimore.²⁵ At a meeting of the Society of Naturalists of the lower Rhine he reported some experiments in which the sheath cells or neurilemma cells which give rise to the bands of cells along the lines of developing nerves were eliminated by cutting out their source at a very early embryonic stage before any nerves what-

²⁵ Harrison, R. G.: "Neue Versuche und Beobachtungen ueber die Entwicklung der peripheren Nerven der Wirbeltiere." Bonn, 1904. Reprinted from Sitzber. d. nied-rhein. Gesellsch. f. Nat. u. Heilk., Bonn, 1904.

ever had developed. He found that these sheath cells arise in the region of the so-called neural crest along with the cells which give origin to the spinal ganglia. By cutting away a thin strip at the back of the embryo, when it is only 3 mm. long, he got embryos to grow which at the end of a week had

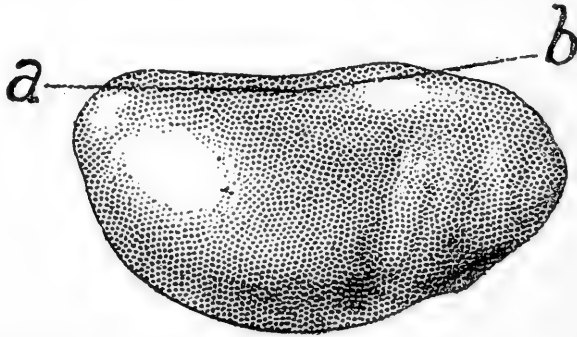


FIG. 22.—Profile view of frog embryo (*Rana esculenta*, 2.7 mm. long) at the stage of operation; the line (a b) indicates the incision. (After Harrison.)

no sensory ganglia or sensory nerves, though they had motor nerves. But the remarkable fact is that these motor nerves, instead of showing cell bands in their course, as under normal

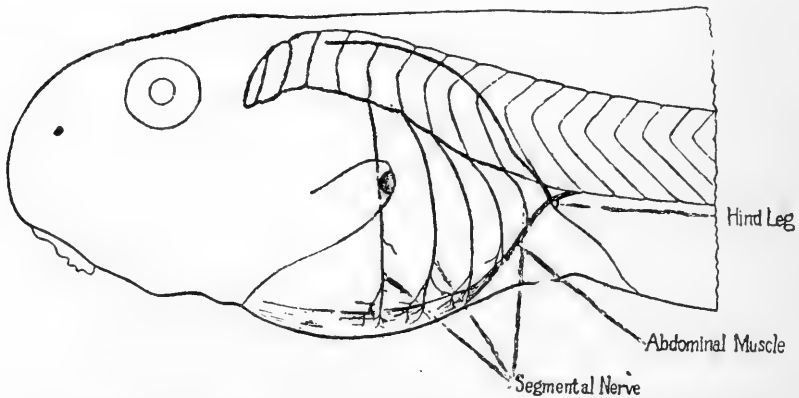


FIG. 23.—Profile view of frog larva (*Rana palustris*, 12 mm. long) after complete resorption of yolk. The relations of the segmental nerves and the primary abdominal muscle are shown. (After Harrison.)

conditions, appeared as naked, non-nucleated fibers which could be traced as such all the way from the spinal cord to the extreme ventral part of the musculature (Figs. 21, 22, 23 and 24). Here, at a blow, the proof was brought that the peripheral spinal nerves may develop in the entire absence of sheath cells.

The first experiments were made on the embryo of *Rana esculenta*; subsequently Dr. Harrison confirmed his results on the embryos of two American species, *Rana sylvatica* and *Rana*

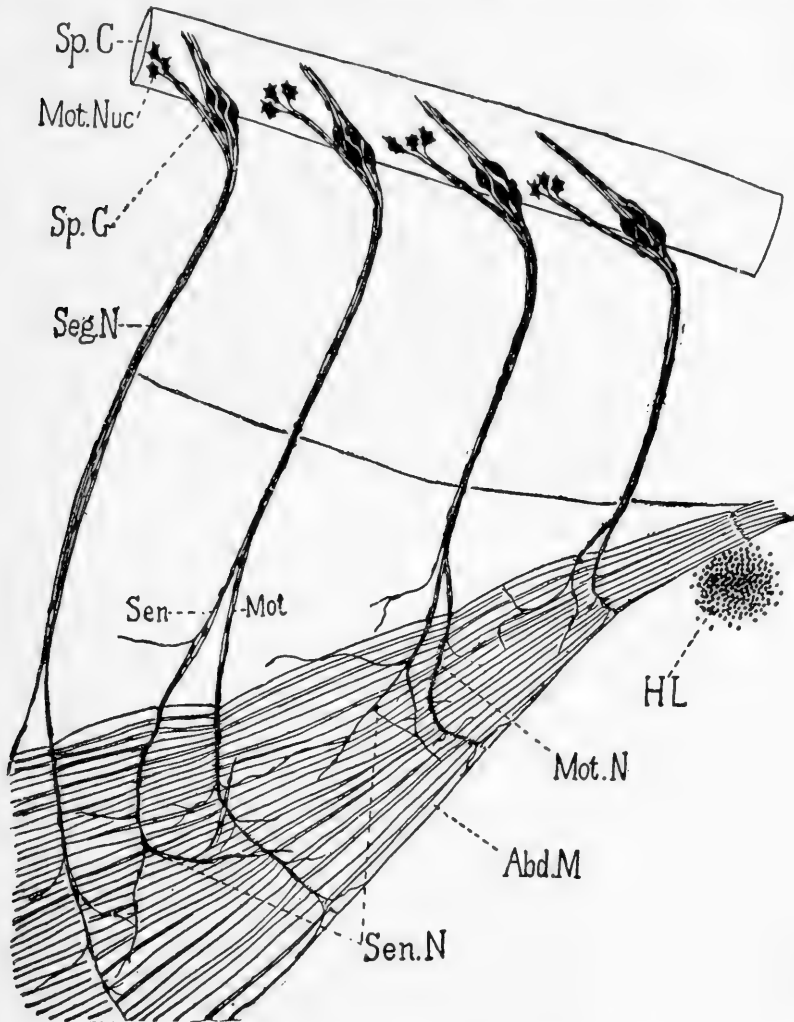


FIG. 24.—Semi-diagrammatic view of the nerves of the abdominal walls of the frog larva (normal specimen). Ab. D, primary abdominal muscle; HL, rudiment of hind leg; Mot. N, motor branch of segmental nerve running in inscriptio tendinea of the primary abdominal muscle; Mot. Nuc., motor nucleus (ventral horn cells) in spinal cord; Seg. N, segmental (spinal) nerve; Sen. N, sensory branch of spinal nerve running to integument outside of muscle; Sp. C, spinal cord; Sp. G., spinal ganglion. (After Harrison.)

palustris. But, true to the principles of the experimental method, Dr. Harrison did not remain satisfied with the proof—he sought the counterproof. As an *experimentum crucis*, it

occurred to him to excise, in these young embryos, the ventral portion of the neural tubes, *i.e.*, to cut away the cells which, according to the doctrine of His, give rise to the axis cylinder

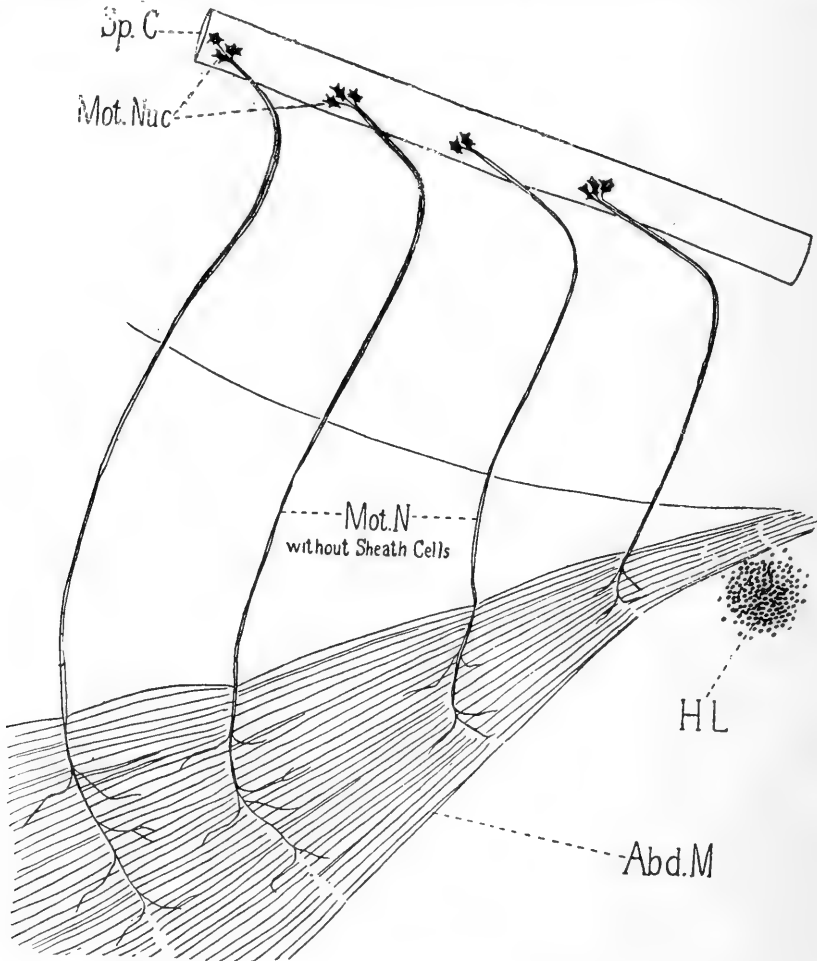


FIG. 25.—Semi-diagrammatic view of the abdominal walls of a frog larva from which the neural crest had been removed, as shown in Fig. 22. Only motor nerves are present, and these consist of axis cylinders without sheath cells. (After Harrison.)

processes of the spinal motor nerves. Leaving the neural crest intact, that is, the region which gives rise to the spinal ganglia and the sheath cells, Harrison argued that if the opposing doctrine that the sheath cells form the motor nerves is true the latter and the sensory nerves should develop normally, even with the anterior horn cells absent. The experiment is one difficult to perform, and a number of attempts failed to give

conclusive results, but Harrison has performed it a number of times successfully. What was the result? The spinal ganglia and the peripheral sensory nerves, with their accompanying sheath cells, developed normally, but not a trace of a spinal

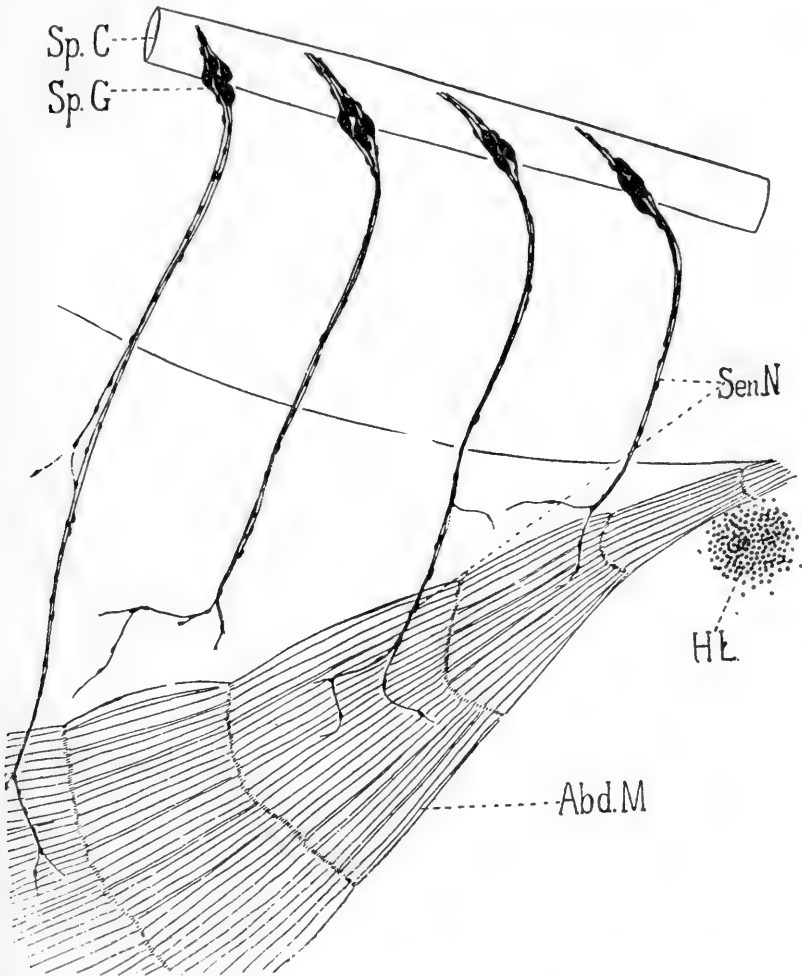


FIG. 26.—Semi-diagrammatic view of the nerves of the abdominal walls of a frog larva from which the ventral half of the spinal cord had been removed at the stage represented in Fig. 22. Absence of the purely motor rami, which normally run in the inscriptiones tendineæ. (After Harrison.)

motor nerve appeared in the region operated on, nor did the sheath cells form bands where the motor nerves normally appeared (Fig. 25). The proof, then, has most brilliantly been brought that the fibers of the motor nerves are processes of the

anterior horn cells, that these processes can extend a long distance from the spinal cord to the muscles in the entire absence of sheath cells, and that the sheath cells are incapable of building these fibers by themselves. If any one could have given me my choice of doing successfully any single piece of experimental work that has been done in neurobiology since 1891, I should unhesitatingly have chosen this. It is an experimental research of the first order, one which, if confirmed, will always redound greatly to the credit of anatomic science in America.

But even should it have been made out that the neuron is pluricellular in its origin, the anatomic unit, which Waldeyer called the neuron, would still have existed. It would have been an organ then, rather than a single cell.

I should like to go on to the newer work, dealing with the origin of the connection between ganglion cell and end organ, into the researches supporting the free outgrowth theory, on the one hand, and the persistent cell-bridge on the other, but I have already exceeded the time allotted to a Harvey lecture and must be content with referring you to Harrison's forthcoming article ²⁶ in which these matters are discussed more ably than I could deal with them.

And now as to the validity of the neuron conception and of the various attached neuron doctrines I shall leave you to judge for yourselves. I am glad if in this hour I have been at all successful in putting the actual facts as known at present before you or in encouraging you to hold an open mind regarding theories where facts are wanting.

²⁶ Harrison, R. G.: Further Experiments on the Development of Peripheral Nerves, *Am. J. Anat.*, Balt., 1906. v. 121-132.

FATIGUE *

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IT is a striking principle of biology that the activity of living substance tends to inhibit its further activity. Carried to a moderate degree, this inhibition leads to the self-preservation of the living substance—to an extreme degree, to its self-destruction. The characteristics of this inhibition, its accompanying phenomena within the organism and the causes that lead to it, form the subject of the present lecture. Fatigue is a comprehensive term, comprising, in its simple form, the functional state of the organism and its constituent parts after activity. Lying on the border zone where the physiologic and the pathologic meet, it reaches far into both and obscures the division lines between them. When present in slight or moderate degree, it limits achievement, but is easily recovered from; when excessive or an accompaniment of disease, it forms a serious condition, which, if in man, the medical practitioner must meet and combat.

Fatigue is a universal biologic phenomenon. The activity, however slight, of living substance, wherever found, reveals its beginnings. While it has been studied chiefly in the muscular and nervous tissues, it has been pointed out elsewhere, and our recognition of its signs is certain of being extended by future study. The chief sign is, in a word, depression—depression of irritability, wherein a given stimulus calls forth a response of less intensity than before; and depression of the total capacity for work, whatever the intensity of the stimulus; its early stages may show, however, a temporary heightened irritability and an apparent, but not real, heightened capacity for work. There are many other signs, recognizable in individual cases.

* Lecture delivered February 3, 1906.

Owing to the unequalled opportunity of applying to the study of muscular activity the exact methods of the physicist and the chemist, the phenomena of muscular fatigue are known more exactly than those of other tissues. Leaving aside for the present the chemical as causative of the physical phenomena, we may consider the latter first—and here the pioneer was Helmholtz,¹ to whom more than to any other in its long history scientific medicine is indebted for exactness in method. Let us assume a voluntary muscle, of either a cold-blooded or a warm-blooded animal, either a lower animal or man, either within the body with the circulation and nervous supply intact or (though this has not yet actually been proved for man) removed from the body. Let such a muscle be stimulated by a series of single artificial stimuli of equal intensity, regularly repeated and applied either directly to the muscle itself or indirectly through the mediation of the nerve, and let the muscle perform mechanical work, such as the lifting of a certain load. We may then observe the following phenomena (Fig. 1): The degree of shortening of the muscle during each contraction increases for a considerable time; hence the height to which the load is lifted or the amount of work that is performed is gradually increased. Later the reverse occurs—the shortening decreases, reaches its original amount, falls below it, and disappears slowly and very gradually, the muscle becoming incapable of performing further work unless a stronger stimulus or a lighter load be employed, or a period of rest be allowed to intervene, or the chemical composition of the muscle be artificially altered in a suitable manner. The irritability of the muscle at first increases and later decreases; its total capacity for performing work begins to decrease at the beginning of the experiment.

At the same time that the spatial changes are going on the time relations of the muscular action are also changing. Here, strangely enough, the muscle of the cold-blooded animal behaves differently from that of the warm-blooded one. In the former,

¹ Helmholtz: Müller's Arch. f. Anat., Phys., u. wiss. Med., 1850, p. 324; 1852, p. 212.

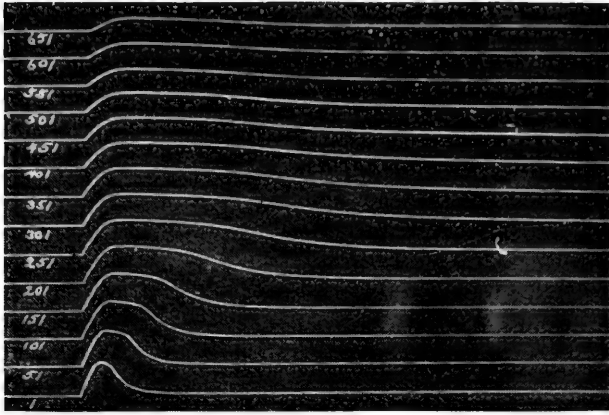


FIG. 1.—Record of fatigue of the frog's gastrocnemius muscle. The numbers signify contractions.

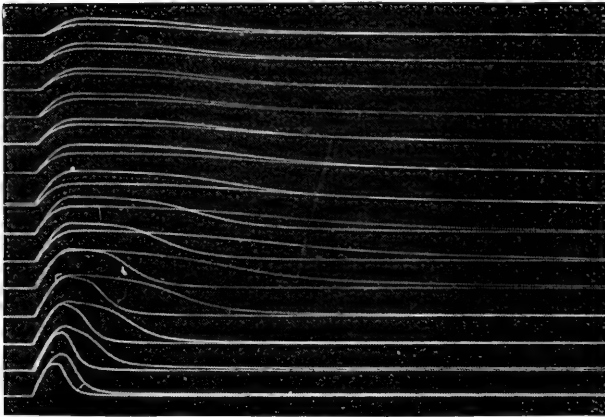


FIG. 2.—Record of fatigue of companion gastrocnemius muscles of the frog, one normal, the other under the influence of sarcolactic acid. The longer, or, in the later contractions, the lower curves, are those of the poisoned muscle. Every fiftieth contraction is recorded.

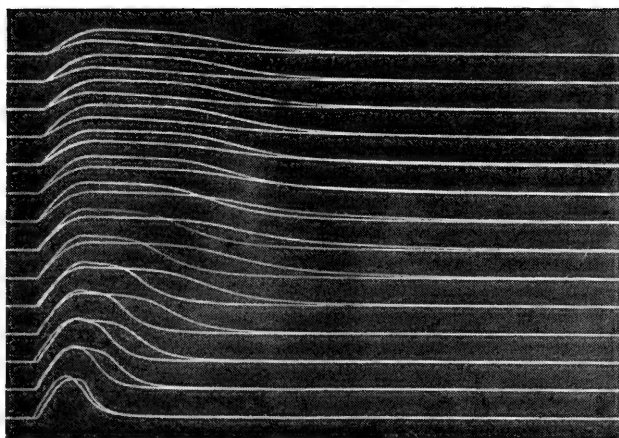


FIG. 3.—Record of fatigue of companion gastrocnemius muscles of the frog, one normal, the other, under the influence of mono-potassium phosphate. The longer, or, in the later contractions, the lower curves, are those of the poisoned muscle. Every fiftieth contraction is recorded.

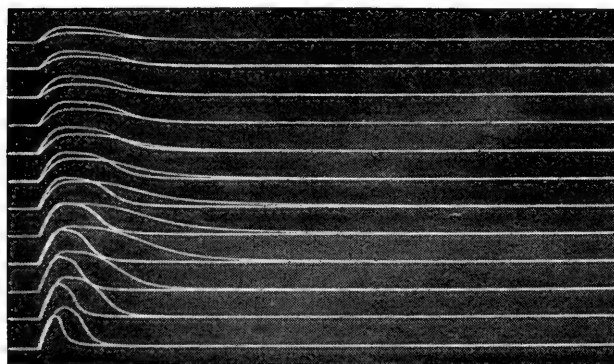


FIG. 4.—Record of fatigue of companion gastrocnemius muscles of the frog, one normal, the other under the influence of carbon dioxid. The longer, or, in the later contractions, the lower curves, are those of the poisoned muscle. Every fiftieth contraction is recorded.

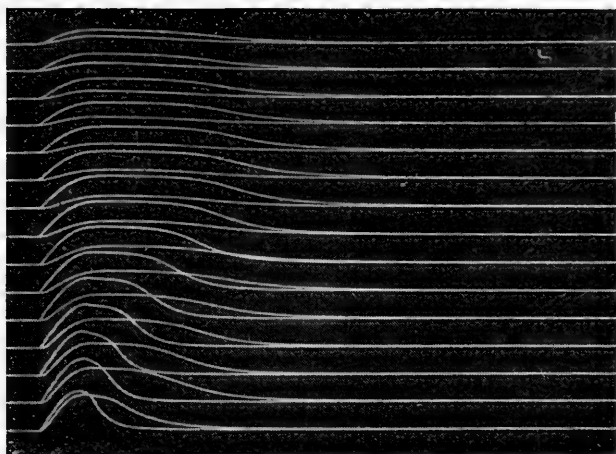


FIG. 5.—Record of fatigue of companion gastrocnemius muscles of the frog, one normal, the other under the influence of β -oxybutyric acid. The longer, or, in the later contractions, the lower curves, are those of the poisoned muscle. Every fiftieth contraction is recorded.

almost from the first, the duration of each twitch begins to lengthen, the whole physiologic process slows, and this reaches large proportions even while the irritability is increasing. The slowing continues for a long time, and only when signs of exhaustion begin to appear does each twitch require a somewhat shortened time for its performance, although the duration is still far greater than at first. The slowing in activity is shared by both contraction and relaxation, but chiefly by the latter, although the degree in which each participates in the phenomenon differs in the muscles of different species. By reason of its existence, by reason of the continuance of the muscle in a contracted state, the organ is less capable of performing work, and, unless the successive stimulations occur at rather long intervals, it usually passes sooner or later into a pronounced and persistent contracture, from which it gives feeble twitches.

In warm-blooded animals, man included, there appears to be no analogous slowing of either contraction or relaxation, while in the later stages of the experiment, when the lifting power is feeble, there may be even a quickening. I have been able to show that this marked difference between the two groups of animals is independent of temperature; it persists even when the cold-blooded muscle is warmed to mammalian temperature, and the warm-blooded muscle is cooled, and it constitutes a real physiologic difference.² The cold-blooded or poikilothermal animal, which lacks a nervous mechanism that regulates its body temperature, represents the more primitive type, from which the homothermal condition has become evolved. It is not surprising that in the course of this evolution the muscle has dispensed with a process which distinctly hampers its activity, and has thus become a much more efficient machine.

Here a word of caution may be necessary. Do not jump to the conclusion, because of what I have said, that the voluntary contraction of a man's muscle, when fatigued, is fully as rapid as or more rapid than when in a fresh condition. We know

² Lee: Arch. f. d. ges. Phys., 1905, vol. cx, p. 400.

from experience that this is not so, that we work more slowly when weary. I have been dealing so far with single contractions, not with voluntary contractions. The latter are always tetani, composed of single contractions fused together. The duration of the single contraction of the human muscle, which can be obtained only by an artificial stimulus, is not lengthened in fatigue; the voluntary contraction may be slowed. More than twenty years ago the Italian physiologist, Mosso,³ devised the important apparatus called the ergograph, and by its means began the long series of studies of voluntary contractions in man, which has made the Turin school famous, and has immeasurably extended our knowledge of fatigue in living human beings. Treves,⁴ one of Mosso's own pupils, Franz,⁵ Hough,⁶ Storey⁷ and others have shown that the original form of the ergograph is defective, and have so perfected the instrument that it bids fair to become an important clinical aid in diagnosis. An ergographic record usually consists of a series of curves of momentary contractions, at regular intervals, of certain finger muscles, either one or more, a known weight being lifted or a spring of known tension being stretched. Such a record exhibits in fatigue a gradual diminution of the lifting power of the muscle, the rate and regularity of the diminution varying with individuals. The single contractions of human muscle, induced by artificial stimuli, directly applied, may be recorded by the same instrument.

In the course of the experiments that I have quoted, it may justly be said that fatigue begins with the first contraction—the

³ Mosso: *Arch. f. Anat. u. Phys., Phys. Abth.*, 1890, p. 89; *Arch. ital. de Biol.*, 1890, vol. xiii, p. 123; "La Fatica," Milano, 1891; English translation: "Fatigue," New York, 1904.

⁴ Treves: *Arch. ital. de Biol.*, 1898, vol. xxix, p. 157, vol. xxx, p. 1; *Arch. f. d. ges. Phys.*, 1899, vol. lxxviii, p. 163; 1901, vol. lxxxviii, p. 7.

⁵ Franz: *Amer. Jour. of Phys.*, 1900, vol. iv, p. 348.

⁶ Hough: *Amer. Jour. of Phys.*, 1901, vol. v, p. 240.

⁷ Storey: *Amer. Jour. of Phys.*, 1903, vol. viii, p. 355.

muscle is less capable of work by reason of this contraction. It is convenient to set apart the late stages as the period of exhaustion, although the beginning of such a period is not marked by distinctive physical phenomena. If at any stage the muscle be irrigated by a stream of fresh blood, by Ringer's solution, or even by an indifferent isotonic solution of sodium chlorid, or, what is less efficient, although in some degree effective, if it be allowed simply to rest, the physiologic pendulum tends to swing back, the irritability and the total capacity for work increase, and physiologically the organ is pushed back to an earlier stage of the fatigue process; in other words, the muscle is in some degree restored.

The term, muscular fatigue, requires a word of explanation, for it has been shown by various investigators, including Waller,⁸ Abelous,⁹ Santesson,¹⁰ and Joteyko,¹¹ that when muscle in fatigue ceases to respond to stimuli sent to it through its nerve, it is still capable of contracting on direct stimulation. Their inference from this fact is that the motor nerve endings within the muscle are the first part of the mechanism to succumb. This inference is probably justified; the nerve endings are probably more susceptible to fatigue than the protoplasm of the muscle cells, and hence the muscle protoplasm itself within the organism probably never reaches the stage of profound exhaustion.

In the study of fatigue the nervous system has occupied a curious position. For while it has long been thought that the brain and spinal cord are, of all parts of the organism, the most susceptible to fatigue, it has been known for more than twenty years that the nerve fiber is extremely resistant. Although Bernstein,¹² in 1877, concluded that the nerve is less easily

⁸ Waller: *Brit. Med. Jour.*, 1885, vol. ii, p. 135; 1886, vol. ii, p. 101; *Brain*, 1891, vol. xiv, p. 179; *Jour. of Phys.*, 1896, vol. xix, p. 1.

⁹ Abelous: *Arch. de Physiol.*, 1893, p. 437.

¹⁰ Santesson: *Skand. Arch. f. Phys.*, 1895, vol. v, p. 394.

¹¹ Joteyko: "Fatigue," *Richet's Diet. de Phys.*, Paris, 1904.

¹² Bernstein: *Arch. f. d. ges. Physiol.*, 1877, vol. xv, p. 289.

fatigued than the muscle, the Russian physiologist, Wedenskii,¹³ was the first to suggest, in 1884, that the nerve may possibly perform its functions altogether without fatigue. Wedenskii stimulated the nerve at a distant point and blocked the passage of the nervous impulses before they reached the muscle by keeping an intermediate portion of the nerve in a constant state of anelectrotonus by means of a polarizing current. At the end of six hours he found the nerve still as active as at first. Maschek¹⁴ obtained a similar result at the end of twelve hours, and, moreover, confirmed the general discovery by substituting ether for a polarizing current. Brodie and Halliburton¹⁵ blocked the impulses by cooling the splanchnic, Bowditch¹⁶ and Durig¹⁷ by using curare with a motor nerve, Szana¹⁸ by atropin with the cardiac vagus, Lambert¹⁹ also by atropin with the secretory fibers of the chorda tympani, while Wedenskii,¹³ Hering,²⁰ Maschek,¹⁴ Edes²¹ and Waller⁸ studied the current of action during long periods of activity. These investigators, working on both cold-blooded and warm-blooded animals, agree in maintaining the extreme resistance of the nerve to fatigue, their experiments continuing in some cases for fifteen hours. Objections have been brought against some of them, notably those in which the persistence of the negative variation is the indicator of indefatigability, it having been shown that negative variation persists even after other evidences of vital action have ceased; nevertheless, the main principle is well

¹³ Wedenskii: *Centbl. f. d. med. Wiss.*, 1884, vol. xxii, p. 65.

¹⁴ Maschek: *Sitzungsb. der Wien. Akad. Math.-Naturwiss. Cl.*, 1887, vol. xcv, No. 3, p. 109.

¹⁵ Brodie and Halliburton: *Jour. of Physiol.*, 1902, vol. xxviii, p. 181.

¹⁶ Bowditch: *Jour. of Phys.*, 1885, vol. vi, p. 133.

¹⁷ Durig: *Centbl. f. Phys.*, 1901, vol. xv, p. 751.

¹⁸ Szana: *Arch. f. Anat. u. Phys., Phys. Abth.*, 1891, p. 315.

¹⁹ Lambert: *Compt. rend. de la Soc. de Biol.*, 1894, p. 511; "La Résistance des Nerfs à la Fatigue," Paris, 1894.

²⁰ Hering: *Sitzungsb. d. Wien. Akad. Math.-Naturwiss. Cl.*, 1884, vol. lxxxix, No. 3, p. 137.

²¹ Edes: *Jour. of Phys.*, 1892, vol. xiii, p. 431.

supported. Garten²² seems, however, to have demonstrated some measure of fatigue in the non-medullated fibers of the olfactory nerve of the fish: by means of continued stimulation the current of action, as measured by the capillary electrometer, becomes diminished in extent; after a pause it increases. Fröhlich,²³ too, believes that he has demonstrated fatigue in a frog's nerve when in partial asphyxiation. On the chemical side no decisive experimental evidence has been brought forward. While Funke²⁴ and Ranke²⁵ find acid in nerves after strong general tetanus, there is no proof that it is formed *in situ*. Waller's²⁶ inference of the production of carbon dioxid in tetanized nerves from the similarity of their electrical phenomena to those of nerves artificially placed under the influence of carbon dioxid seems hardly justified from such slight evidence. Our conclusion must, therefore, be, and with this we shall find general agreement, that, while nerve is probably not indefatigable, it is extremely resistant to fatigue in comparison with other peripheral tissues, and that, although nerve protoplasm is not an exception to the general biologic law according to which katabolic changes occur during activity, such changes here present are either minute or, more probably, are at once compensated for by adequate anabolism.

But the demonstrated resistance of nerve fibers could not easily shake the firm belief of physiologists in the extreme susceptibility to fatigue of the central portion of the nervous system. It has long gone without dispute that in prolonged activity the brain and spinal cord succumb first, and thus the

²² Garten: Beiträge zur Phys. der marklosen Nerven," Jena, 1903.

²³ Fröhlich: Zeits. f. allg. Phys., 1904, vol. iii, p. 468.

²⁴ Funke: Ber. Sächs. Akad., 1859, p. 161; Müller's Arch. f. Anat., Phys., u. wiss. Med., 1859, p. 835.

²⁵ Ranke: "Tetanus," Leipzig, 1865; Centbl. f. d. med. Wiss., 1868, vol. vi, p. 769; "Die Lebensbedingungen der Nerven," Leipzig, 1868.

²⁶ Waller: "Lectures on Physiology; On Animal Electricity," London and New York, 1897; Phil. Trans. of the Roy. Soc., B. 1897, vol. clxxxviii, p. 64.

exhaustion of the peripheral tissues is prevented. The nerve center has been compared to the fuse of an electric circuit, the burning out of which protects the muscle from grievous injury. By most upholders of the neuron theory central fatigue has been referred to the bodies of the nerve cells, in which Hodge,²⁷ Vas,²⁸ Mann,²⁹ Lugaro,³⁰ Eve,³¹ and others have demonstrated histologic changes after activity. According to most of these observers, moderate activity is accompanied by an increase in the bulk of both cytoplasm and nucleus, excessive activity by a decrease in bulk and the appearance of vacuoles in both, and a loss of the substance of the Nissl bodies. While these histologic changes after excessive activity have generally been interpreted as significant of fatigue, there does not exist general agreement as to their mode of origin.

Professor Sherrington,³² a strenuous adherent of the neuron theory, a clear thinker and one of the ablest and most careful experimenters, whose investigations in recent years have widely extended our knowledge of the mode of action of the nervous system, doubts the inferences that have been drawn from the histologic observations and denies central fatigue to the bodies of the neurons. He ascribes great physiologic importance to the structure—if it can be called a structure—that is situated at the point where one neuron comes into functional relation with the next in the series, this structure being the synapse. The synapse is the surface of contact of the two neurons and is potentially a membrane. One of Sherrington's experiments is as follows: Given a certain center within the spinal cord, which

²⁷ Hodge: *Amer. Jour. of Psych.*, 1888, vol. i, p. 479; 1889, vol. ii, p. 376. *Jour. of Morphol.*, 1892, vol. vii, p. 95.

²⁸ Vas: *Arch. f. mik. Anat.*, 1892, vol. xl, p. 375.

²⁹ Mann: *Jour. of Anat. and Phys.*, 1894, vol. xxix, p. 100.

³⁰ Lugaro: *Lo Sperimentale, Sez. biol.*, 1895, vol. xlix, p. 159.

³¹ Eve: *Jour. of Phys.*, 1896, vol. xx, p. 334.

³² Sherrington: "The Spinal Cord," Schäfer's *Text-book of Physiology*, vol. ii, p. 831, New York, 1900; *Proc. of the Brit. Assoc. for Adv. of Sci.*, 1904, *Ergebnisse d. Phys.*, 1905, vol. iv, p. 797; *Jour. of Phys.*, 1906, vol. xxxiv, p. 1.

can be reached by several afferent tracts, and a common efferent tract to a given muscle, he stimulates one of the afferent tracts and records the reflex contractions of the muscle. In the course of time the motor response gives evidence of fatigue. He then turns to another afferent tract and stimulates it. The motor response now appears as strong as at first. Where is located the fatigue from the first stimulation? Not in the nerve fibres, says Sherrington, for they are practically incapable of fatigue; not in the muscle or the body of the motor neuron, for they are common to the two stimulations. Hence it must be at the synapse between the first afferent tract and the motor neuron. Sherrington likens the synaptic membrane to the motor end-plate, the former constituting the safety fuse within the central mechanism, the latter playing a similar rôle within the muscle; while of the two the synapse is the more susceptible.

Other experiments, however, indicate that there is less justification than has commonly been supposed for the idea that the central nervous system fatigues before the muscular system, and lead us to suspect that the reverse is true. Woodworth,³³ for example, finds no perceptible difference in the rate of fatigue when one gastrocnemius is stimulated directly at the same time that the other is stimulated either through the medulla or through a sensory nerve, thus indicating that in such an experiment the nerve center contributes no appreciable amount of fatigue. Mlle. Joteyko,¹¹ of Brussels, destroys the brain of a frog, thus rendering the animal a reflex machine, and exposes both sciatic nerves. On stimulating nerve A, she obtains direct contractions of its gastrocnemius muscle (A) and reflex contractions of the gastrocnemius (B) of the opposite side. She then blocks the passage of the impulses in nerve B by means of either a continuous polarizing current or ether, and continues to stimulate nerve A. When its muscle is exhausted the block is removed from the opposite nerve, and the muscle of that side responds nearly as well as at first to the

³³ Woodworth: N. Y. Univ. Bull. of the Med. Sci., 1901, vol. i, p. 133.

reflex stimulations. The reflex centers thus remain active long after fatigue has placed the muscle *hors de combat*. Joteyko concludes that, compared with the terminal organs, the reflex mechanism of the spinal cord is practically indefatigable.

If this conclusion be true, why may not the same be said of the brain centers? The common belief in the susceptibility of the brain to fatigue is based largely on the presence of sensations of fatigue. With such sensations as a daily experience we are all familiar. They are the psychologic concomitants of physiologic processes; and, since we know that the brain is the seat of the former, it is only natural to believe that the physiologic processes occur there also. We feel tired, and we infer that our brain is tired. For long a controversy has raged regarding the origin of the feeling of effort that accompanies muscular work. Is it central? Does the consciousness of the motor discharge precede actual movement? Does the sense of effort decrease as we continue to labor? In recent years we have learned much regarding the nervous relations of muscle, and the existence of the well-developed muscle sense has been established. Sensory end-organs have become recognized in muscles and tendons, and afferent fibers in muscle nerves; the muscles undoubtedly keep the brain informed of their general condition and of the intensity of their contractions. Along with this advance of our knowledge, it has become generally recognized with Wundt, James, Münsterberg and Baldwin that the feeling of the amount of effort required to make muscles contract is dependent on impulses reaching the psychic centers from the muscles, tendons and joints. The feeling of effort is of peripheral origin. The same is probably largely true of the feeling of fatigue. We are distinctly conscious of the fatigue of our muscles; their tone is diminished; their unusual tension gives us a feeling that they are heavy; it seems more difficult to make them respond to our will, and their response is often painful. Moreover, we are aware that our limbs are swollen, that blood vessels are dilated, and that lymph has accumulated in the intercellular spaces. These are but a few of the sensations. Other tissues add their share of stimuli,

many of them obscure and difficult of analysis and location. The result of the flood of these impulses pouring into the brain is a large complex of sensations, which we call the feeling of fatigue. Experiment also appears to justify the peripheral location of the influences that lead to our feeling. Mosso,³ by means of his ingenious instrument, the ponometer, demonstrates that during a series of voluntary muscular contractions resulting in fatigue the nervous effort to contract gradually increases; in other words, that the curve of nervous effort is the reverse of the curve of muscular performance. Evidence seemingly contradictory to the theory of the peripheral origin of fatigue sensations was contributed by Mosso,³ Lombard³⁴ and Waller,⁸ and for a time had wide acceptance. These investigators claim to have found that when a set of muscles, such as the flexors of the finger, stimulated by volition and lifting a given weight, became incapable of further voluntary contraction, they still responded readily to electrical stimuli applied directly to the muscles themselves. This supposed phenomenon, widely quoted, and at first thought decisive, has been examined critically by Kraepelin,³⁵ G. E. Müller,³⁶ Henri,³⁷ R. Müller,³⁸ Hough,⁶ Woodworth,³³ Storey⁷ and Joteyko,¹¹ and the validity of its proof has been discredited. R. Müller shows, for example, that the muscles that were stimulated volitionally and those that were stimulated electrically were in reality not the same, the former being the *interossei*, the latter the flexors of the finger. Storey has repeated the work with an improved form of ergograph and with the abductor indicis alone, and clearly demonstrates the appearance of peripheral but not central fatigue. In view of these results

³⁴ Lombard: *Arch. ital. de Biol.*, 1890, vol. xiii, p. 371; *Amer. Jour. of Psych.*, 1890, vol. iii, p. 24.

³⁵ Kraepelin: "Ueber die Beeinflussung einfacher psychischer Vorgänge durch einige Arzneimittel," Jena, 1892.

³⁶ Müller, G. E.: *Zeitsch. f. Psych. u. Phys. d. Sinnesorgane*, 1893, vol. iv, p. 122.

³⁷ Henri: *Annal. Psych.*, 1899, vol. v.

³⁸ Müller, R.: *Wundt's Philosoph. Studien*, 1901, vol. xvii, p. 1.

and others, I am inclined to the belief that when we perform continued muscular work, our muscular system fatigues before our central nervous system. Moreover, the same results make it probable that the brain and the spinal cord are, like the nerve fiber, resistant, and they throw a certain measure of doubt on all supposed proofs of central fatigue.

With the general problem in this somewhat uncertain state, what can we say of mental fatigue? That it is a reality can not, of course, be denied. It is characterized pre-eminently by a weakening of the powers of attention and the reproductive phase of memory, and the psychophysical laboratories have shown us in innumerable ways how it manifests itself. To explain it on physiologic principles is not altogether possible in the present state of research. Our present theory interprets it as largely peripheral in origin, and Mosso's³ school has demonstrated, though by imperfect methods, that intense mental work, long continued, such as in the oral examination of many students, diminishes the power of the muscles to respond to direct stimulation, the locus of the fatigue in this case being largely the muscles, as Mosso admits. But to what extent so-called mental fatigue is of peripheral origin we can speak only with caution. We can not deny fatigue to psychic centers, but the intimate relations of central and peripheral fatigue are much in need of exact experimental study.

It is customary to seek the causes of the physical phenomena of fatigue in the chemical changes undergone by the active living substance. Unfortunately, we know too little of these chemical changes. It has been pointed out by von Noorden³⁹ and Levene,⁴⁰ in their illuminating lectures before this Society, that, while the final products of protoplasmic activity are well known, we are sadly ignorant of the intermediate steps between income and outgo. In all tissues during activity substances of value to the organism are broken down and substances of

³⁹ v. Noorden: This volume, p. 18.

⁴⁰ Levene: This volume, p. 73.

little or no value are formed. When men began to speculate, in the light of the scientific chemistry of the nineteenth century, concerning the causes of fatigue, it was perceived that they might be of two kinds: the loss of valuable material essential to activity, and the accumulation of waste products. Naturally the discussion centered on muscle, since many endeavors were being made to seek the source of muscular energy.

Without entering in detail into the interesting history of these endeavors, which are not yet ended, it is sufficient to say that it is now generally recognized that, under ordinary circumstances, the chief source of muscular energy is carbohydrate. Weiss,⁴¹ a pupil of the Viennese physiologist, Brücke, definitely proved in 1871 that a marked diminution of glycogen accompanies muscular activity, and since then many others have demonstrated the same fact in a variety of ways. We might then expect muscular fatigue to be associated with loss of carbohydrate. So far as I am aware, but one research bearing directly on this subject has been made, namely, that of Harrold⁴² and myself, performed several years ago and still unpublished in detail. We allowed cats to fast for several days and, during the latter portion of the period, administered hypodermically considerable doses of phlorhizin, which removes carbohydrate from the body. At the end of an adequate period, when, as the experiments of others have shown, the tissues were practically freed from this substance, the animals exhibited great muscular weakness. They were then killed and contraction records were made by selected muscles, artificially stimulated. It was found that under the influence of the drug the muscles were capable of making only from one-fifth to one-half of the number of contractions of which a normal muscle is capable. We proved that this diminution in working power was not due to a direct specific action of the drug on the muscle tissue. The result may, therefore, be

⁴¹Weiss: *Sitzungsb. d. Wien. Akad. Math.-Naturwiss. Cl.*, 1871, vol. lxiv, No. 2, p. 284.

⁴²Lee and Harrold: *Amer. Jour. of Phys.*, 1900, vol. iv, p. ix.

explained in one or both of two ways: either by the loss of carbohydrate or by the accumulation of pathologic acids, which are now known to be formed both during fasting and under the influence of phlorhizin. That the former, however, was largely responsible for the fatigue seems probable from our further experiment, namely, that the administration of a quantity of dextrose to a phlorhizinized, and thus thoroughly fatigued, animal was followed within a few hours by a considerable return of muscular power. Our results are well supplemented by those of Mosso and Paoletti,⁴³ Harley,⁴⁴ Frey,⁴⁵ Schumburg,⁴⁶ and Hellsten,⁴⁷ all of whom working with the ergograph on human beings have observed an increase of working power and a diminution of fatigue after the ingestion of sugar.

A condition similar to that of our experiment is met constantly in disease. The physical weakness of fevers, of diabetes mellitus, and of many other pathologic states, in which a deranged metabolism exhausts the muscle cells of their proper store of available nutritive material, a physical weakness which is, physiologically, fatigue, is doubtless due in part to the lack of energy-yielding carbohydrate. Concerning a possible relation of the loss of other substances to fatigue, our present knowledge permits us to say nothing.

Ranke²⁵ was the first to investigate, from the standpoint of fatigue, the physiologic action of the products of protoplasmic activity. More than forty years ago he studied the action on frog's muscle of the supposed products of muscular action, namely, lactic acid, kreatin, kreatinin, sugar and carbon dioxid. He found that of all these substances only kreatin and lactic acid markedly depress muscular action, as measured by the strength of the induced current necessary to stimulate and also

⁴³ U. Mosso and Paoletti: *Arch. ital. de Biol.*, 1894, vol. xxi, p. 293.

⁴⁴ Harley: *Jour. of Phys.*, 1894, vol. xvi, p. 97.

⁴⁵ Frey: *Mitth. aus klinik. u. med. Inst. d. Schweiz. Annales suisses des Sci. med.*, 1896, vol. iv, p. 1.

⁴⁶ Schumburg: *Deutsch. milit. Zeits.*, 1896, vol. xxv, p. 337.

⁴⁷ Hellsten: *Skand. Arch. f. Phys.*, 1904, vol. xvi, p. 139.

by the height to which the muscle is able to lift a given load. He therefore designated these two substances as fatigue substances. Carbon dioxid was found to be slightly depressant, but not sufficiently so to be regarded as playing a distinctly fatiguing action. Both lactic acid and kreatin were found to augment nervous activity, and carbon dioxid slightly to depress it. Later Ranke rejected kreatin as a fatigue substance and accepted acid potassium phosphate. Little experimentation in this subject has been done since Ranke's time; but, partly from his results and partly from other considerations, it is now customary to recognize three distinct metabolic products as fatiguing, namely, sarcolactic acid, mono-potassium phosphate (KH_2PO_4) and carbon dioxid, all of which are acid in reaction. The action of these substances on the muscular and the nervous systems has never been fully investigated. My own experiments on muscle, which are not yet completed, have already yielded certain positive results. On account of the ready recognition of the phenomena of fatigue in frogs, I have worked so far with them only, but there is no reason to doubt that the results are equally applicable to mammals. I shall, however, extend the work to the latter. My method is to inject one gastrocnemius muscle with physiologic salt solution and the opposite gastrocnemius with similar solution containing a given quantity of the substance which is to be investigated. After a certain time the muscles are excised and stimulated at regular intervals, and comparative fatigue records of the two are made. In this manner I have studied the physiologic action on excised muscle of the three recognized fatigue substances.

Under the influence of a minute quantity of free sarcolactic acid the frog's gastrocnemius, regularly stimulated, presents a striking series of contractions (Fig. 2). The first contraction curve is usually higher and longer than that of the normal muscle. In successive curves this increased height is maintained for a certain time, but later gives place to the normal height and then to one still lower. From the first curve onward the length rapidly increases in proportion to the normal length until the increase has become very marked; later it

slightly decreases. The curves show that in the poisoned muscle there is at first increased and then decreased lifting power, increased and then decreased duration of action in comparison with the non-poisoned muscle. At first sight the effect might be interpreted as due to a beneficial action of the acid. But such an interpretation is a superficial one. More careful observation shows that the fatigue series of the poisoned muscle is like that of the non-poisoned one, if the early contractions of the latter be left out of account. The first contraction curve of the poisoned muscle is not unlike the fiftieth or seventy-fifth of the non-poisoned, the fiftieth of the former like the one-hundredth or the one-hundred-and-fiftieth of the latter, and so on. In other words, the acidified muscle is already fatigued at the beginning of the series, and with stronger doses of the acid the fatigue is more pronounced. Sarcolactic acid is truly named a fatigue substance. Whether, however, the acid in the free state exerts its depressant action is unknown. While it is conceivable that immediately on its formation it may act on the protoplasm of the cells in which it arises, it occurs in the blood not free but as a neutral salt, probably of potassium. I find the action of potassium sarcolactate to be indistinguishable, qualitatively, from that of the free acid, although a stronger solution of the former is required to produce the same quantitative effect.

After the injection of a few cubic centimeters of a one-fifteenth-gram molecular solution of mono-potassium phosphate, the poisoned gastrocnemius shows the following phenomena (Fig. 3): The first contraction of the series is usually closely similar to the first contraction of the normal muscle; very soon, however, the poisoned muscle shows signs of fatigue, and then rapidly passes through the sequence of events which we have already recognized as characteristic of the gastrocnemius. The poisoned muscle becomes fatigued so rapidly that at last it is scarcely able to lift the weight, while its normal mate is still in excellent working condition. Mono-potassium phosphate, therefore, diminishes the working power and is distinctly fatiguing. I have made some attempt by means of parallel

experiments with mono-sodium phosphate, which I find also fatiguing, to discover whether the action of the potassium salt is due to its potassium or to its hydrogen ions. So far as my experiments have gone they indicate that the two substances share in the general effect; the latter seems not to be an acid action solely. The depressant influence of the potassium ions on striated muscle is not unexpected in view of their inhibitory and relaxing effects on the heart, which have been observed by Howell ⁴⁸ and will be discussed by him in a later lecture before this Society.

Carbon dioxid, when injected into the circulation, produces a result in general similar to that of sarcolactic acid or mono-potassium phosphate (Fig. 4). Like them, it is markedly fatiguing.

My results seem to show a certain qualitative physiologic difference between mono-potassium phosphate on the one hand and sarcolactic acid and carbon dioxid on the other, though at present I would not assert this with positiveness. While the muscle under the influence of the salt seems at first not fatigued, but runs through its fatigue course rapidly, the muscle poisoned by sarcolactic acid or carbon dioxid appears fatigued at once, the first contraction being usually slower than the first normal contraction. Whether this apparent difference, which at best is only secondary, proves true or not, it is evident that sarcolactic acid, both free and combined, mono-potassium phosphate and carbon dioxid are fatiguing to muscle. Though I have not yet investigated their action on the central nervous system, the legitimacy of the term fatigue substance is abundantly proved. The organism produces normally in the course of its activity a number of acid substances which tend to inhibit further activity. Fatigue is due in great measure to the depressant action of these toxic products of metabolism on the body tissues, particularly on the muscular system, and the sensation of fatigue is in large part the psychic manifestation of the recognition of this depressant action.

⁴⁸ Howell: Amer. Jour. of Phys., 1898, vol. ii, p. 47; 1901, vol. vi, p. 181; 1906, vol. xv, p. 280.

My friend and colleague, Professor Curtis, whose invaluable studies of the early history of medicine often correct our false perspective and distorted judgments by showing us how our modern discoveries were foreshadowed long before, has called my attention to the following passage from the Hippocratic writings,⁴⁹ which I am permitted to quote in his own translation:

As to the varieties of fatigue which the body may experience, matters stand as follows: Untrained men are fatigued by every exertion, for no part of the body has been exercised at any kind of effort. Trained bodies are fatigued by unaccustomed forms of exertion and also by their accustomed exercises pushed to excess. Such are the forms of fatigue, and their potency takes effect as follows: The untrained have moist flesh, and when they exert themselves the body becomes heated and they yield the product of liquefaction in abundance. Of this, whatever is sweated out or purged away with the breath causes no trouble except to so much of the body as has undergone the unusual depletion, but whatever remains of the product of liquefaction causes trouble, not only to the unduly depleted part of the body, but also to whatever part receives the liquid in question, which is not akin to the body, but hostile. It does not so much affect the fleshless parts of the body, but rather the fleshy, causing trouble until it takes itself off. Inasmuch as it fails to move about, it keeps quiet and becomes heated, itself and what accrues to it; and if there come to be abundance of this separated stuff it may overpower the healthy parts so that the whole body becomes heated likewise and [the stuff in question] may cause severe fever.

These ideas, recorded twenty-three hundred years ago and in language to us quaint, are strangely prophetic. To Hippocrates and his contemporaries, as to us, excessive activity of a part of the body may cause general fatigue. To them the result is due to the "product of liquefaction," which is "hostile" to the body and affects chiefly the "fleshy" parts, there "causing trouble until it takes itself off." To us the result is due to "fatigue products," which are "toxic" to the body and affect chiefly the muscles, there "causing trouble" until they are either excreted or rendered innocuous by chemical

⁴⁹ Hippocrates: "Oeuvres complètes d'Hippocrate. Traduction nouvelle avec le texte grec en regard," E. Littré, vol. vi, p. 582, Paris, 1849.

change. Those same Greeks, however, whose speculations came so near the truth in one regard, knew not even the functions of the muscles or the other parts about which they were writing!

It is not improbable that future research will discover other fatigue substances besides those that I have named. Mention should here be made of the claim of Weichardt,⁵⁰ working in Zuntz's laboratory in Berlin, to have isolated from fatigued muscles a true toxin, of a chemical and physical nature like bacterial toxins, which, when introduced in minute quantity into the body, is capable of giving rise to the phenomena of fatigue. Weichardt further claims to have obtained by the usual methods of the bacteriologists an antitoxin endowed with the power of neutralizing the fatiguing properties of the toxin. So sweeping a discovery needs confirmation before it can be accepted. But it is hardly credible that the few substances that I have mentioned should prove to be, of all the links in the long metabolic chain, the only substances that are depressant to protoplasmic activity. It is the intermediate substances that we must watch especially. Autolysis opens up also a new field full of interest in this connection.

Concerning the production of fatigue substances by the central nervous system, very little is known. There is no certain evidence that sarcolactic or other organic acid is produced by nervous activity. In view of the striking fact which Macallum⁵¹ has brought forward by means of his careful micro-chemical methods, namely, that potassium is not to be found in the neuron, we can not believe that potassium salts play any rôle in nervous fatigue. Carbon dioxid is, however, present, and to it has been ascribed by Verworn⁵² an important share in the phenomenon in question. Yet Hill and Nebarro⁵³ have

⁵⁰ Weichardt: Münch. med. Wochsch., 1904, pp. 12 and 2121; 1905, p. 1234; 1906, pp. 7 and 1701.

⁵¹ Macallum: Jour. of Phys., 1905, vol. xxxii, p. 95.

⁵² Verworn: Arch. f. Anat. u. Phys., Phys. Abth., Suppl., 1900, p. 152.

⁵³ Hill and Nebarro: Jour. of Phys., 1895, vol. xviii, p. 218.

investigated the content in carbon dioxid of the venous blood which has passed through the muscles and that which has passed through the brain during both rest and activity, and find the systemic blood to contain between two and three times as much of this gas during rest as the cerebral venous blood contains, and from three to seven times as much during activity. They conclude that the metabolism of the muscles is, during rest, twice to three times as great as that of the brain, and that during activity muscular metabolism increases enormously in comparison with the metabolism of the brain—a further suggestion that the fatigue of the muscles is of pre-eminent importance relative to that of the central nervous system.

That intense mental activity is capable, however, of giving rise within the body to profound chemical changes is proven by the not unfrequent occurrence of such cases as the following, this striking instance of which was recently related to me by its observer, a well-known medical authority: A nursing mother was subjected for a few minutes to intense fright. Her child, after taking the mother's milk some three or four hours later, was attacked by convulsions. But there is no evidence that the toxic substance in the mother's milk was produced in the brain cells. Indeed, the probability is that the deranged metabolism was localized elsewhere, an indirect result of the nervous shock.

The action of fatigue substances is not confined to the tissues in which they arise. The excessive activity of one tissue is capable of causing fatigue to appear in others. We all know that fatiguing muscular work diminishes our brain power, and I have already referred to the experiment by which Mosso's school has demonstrated that after intense mental labor the muscles are less capable of performing work on direct stimulation. Thus, localized activity is capable of producing general fatigue, a fact which is often overlooked in our daily life. The explanation of this is afforded by Mosso's ⁵⁴ well-known experiment: A dog was fatigued by long continued running; his

⁵⁴ Mosso: *Verhand. d. internat. med. Cong. zu Berlin, 1890*, vol. ii, pt. 2, p. 13.

blood was then transfused into the vessels of a second dog, from which an equivalent amount of blood had been withdrawn, with the result that the second dog exhibited the usual phenomena of fatigue. The blood had evidently become charged with the fatigue substances produced in the muscles, and thus they were able to reach all parts of the body. Geppert and Zuntz⁵⁵ and others have demonstrated that in muscular work resulting in general fatigue the alkalinity of the blood, as determined by titration methods, is markedly diminished. Zuntz⁵⁶ has pointed out that in such a condition the circulatory and the respiratory organs are first affected, later the digestive and the urinary organs. Geppert and Zuntz⁵⁵ and Lehmann⁵⁷ have demonstrated that the increased action of the respiratory center in muscular work is probably caused by the stimulating action of the acid fatigue substances arising in the muscles. It seems to be a fact that in general fatigue the coagulation of the blood is hastened, while according to Manca⁵⁸ the red corpuscles break down less rapidly than before. Ceni⁵⁹ claims that the bactericidal power of the blood is diminished in brief and increased in prolonged muscular fatigue. Salvioli⁶⁰ finds the salivary and the gastric glands to secrete less during intense muscular fatigue, and the gastric juice to lose in acidity and in digestive power. Although exact researches are here needed, there are probably few physiologic functions that are not affected unfavorably by the prolonged and excessive activity of the muscular and the nervous systems. In such a condition the normal action of the tissues may easily give place to pathologic action, as is illustrated by the fever resulting from over-exertion. Fatigue undoubtedly diminishes the resistance of the tissues to bacteria and also predisposes the individual to

⁵⁵ Geppert and Zuntz: *Arch. f. d. ges. Physiol.*, 1888, vol. xlii, p. 189; 1895, vol. lxii, p. 295.

⁵⁶ Zuntz: *Le Bull. Med.*, 1903, vol. xvii, p. 778.

⁵⁷ Lehmann: *Arch. f. d. ges. Phys.*, 1888, vol. xlii, p. 284.

⁵⁸ Manca: *Arch. ital. de Biol.*, 1895, vol. xxiii, p. 317.

⁵⁹ Ceni: *Arch. ital. de Biol.*, 1893, vol. xix, p. 293.

⁶⁰ Salvioli: *Arch. ital. de Biol.*, 1892, vol. xvii, p. 248.

attacks from diseases other than bacterial. Specific investigation in its relation to pathologic conditions is, however, sadly lacking.

Nevertheless there is one subject in pathology concerning which we can speak in more than general terms. The fact that certain acid substances are depressants has a far wider application than merely in the causation of physiologic fatigue. Pathologists have recognized in recent years as a widespread phenomenon the production in quantity of acids, which load the blood and the tissues and give rise to the condition known as acid intoxication. This condition has been demonstrated notably in diabetes mellitus, fevers, carcinoma, anemia, acute yellow atrophy of the liver, phosphorus and arsenic poisoning, arthritis deformans, various disorders of digestion, and inanition; and the acids which are involved are such as exist normally only in small quantities, if at all, and are intermediate products of metabolism, the results of incomplete oxidation. In nearly all, if not all, of the diseases mentioned, among the prominent symptoms are marked depression and ready fatigability. Zenoni⁶¹ has made ergographic tracings from diabetics and finds advanced cases capable of a very small amount of muscular work, a limited number and a small amplitude of contractions, not rarely accompanied by contracture, and an early onset of fatigue. The facts of the toxic theory of fatigue suggest that we have in the pathologic acids and the depression and ready fatigability a relation of cause and effect.

It is a well-known fact that the injection of these acids into living organisms is followed by the symptoms that follow their spontaneous formation within the body—artificial acid intoxication resembles spontaneous acid intoxication. I have been able to demonstrate on muscle the fatiguing action of one of the pathologic acids, namely, β -oxy-butyric acid, which is common in diabetes mellitus (Fig. 5). A frog's gastrocnemius under the influence of β -oxy-butyric acid reacts not unlike a muscle under the influence of sarcolactic acid or carbon

⁶¹ Zenoni: *Il Policlinico, Sez. med.*, 1896, vol. iii, p. 538.

dioxid. There is pronounced fatigue from the beginning, a curve of contraction that is high and long at first and becomes lower and longer as the experiment proceeds, and an early onset of exhaustion. Such a muscle is already in a fatigued condition, even before the series of stimulations has begun. Such a muscle within the body would undoubtedly give rise to fatigue sensations. To what extent within the body the free pathologic acid acts directly on the muscles is a problem like that presented by sarcolactic acid and is yet unsolved. It seems probable that the muscles are one of the places of origin of the pathologic acid, and it is possible that the free acid exerts its depressant action on the cells in which it is formed. It seems to occur in the blood of diabetics, however, not free, except in the most extreme cases, but in combination with certain metals, such as sodium, potassium and ammonium. I have investigated the salts so formed, namely, sodium, potassium and ammonium oxybutyrates, which, it should be emphasized, are not acid in reaction, and I find their action on muscle to be similar qualitatively to that of the free acid. Quantitatively they differ among themselves, the potassium salt being the most powerful. While, therefore, the free acid may possibly act as a fatigue substance to the cells in which it originates, its salts may act through the blood on distant cells and give rise to general fatigue, physical depression and ready fatigability. My results do not seem to require the acceptance of Minkowski's ⁶² hypothesis, based partly on his own observations and partly on those of Walter,⁶³ that the attachment of the sodium of the blood forces the carbon dioxid, continually produced, to remain in the tissues and that the fatigue is then referable to it, rather than to the pathologic acid or its salts. My present inclination, however, is toward the belief that all these substances are causative. The salts certainly are so, and the beneficial effects which are known to follow the administration of

⁶² Minkowski: Naunyn's Mitth. aus. d. med. Klinik zu Königsberg, 1888, p. 174.

⁶³ Walter: Arch. f. exp. Path. u. Pharm., 1877, vol. vii, p. 148.

alkalies, such as sodium bicarbonate, seem to demand free acid and perhaps carbon dioxid also as causative agents. However this may be, I wish to emphasize my main contention, namely, that in acid intoxication the tissues of the body are in a state wholly analogous to the state of fatigue, in so far as the latter is due to toxic substances. The facts that the fatigue products are produced in the one case normally and in the other pathologically and that they differ in composition in the two cases are altogether secondary. Their physiologic effects are the same. Heretofore attention has been directed chiefly to the extreme effects of the pathologic acids, as, for example, the production of diabetic coma. We should not, however, forget that long before these extreme effects are manifested, the same causes are producing evil, if less obvious, phenomena and rendering the cells less capable of their proper functions.

Not all cases of fatigue accompanying pathologic conditions are, however, due to excessive production of acid. In diabetes itself we have an excellent example of the twofold cause. The body is not only intoxicated by acid, but carbohydrates are wanting, and I can cite no more typical instance of disease in which fatigue may be traced to its two recognized causes. The two causes are also present in fevers and inanition, and doubtless in other pathologic conditions, though here investigation is needed.

Several investigators, especially Abelous and Langlois⁶⁴ and Albanese,⁶⁵ have studied the relation of the suprarenal bodies to fatigue. They find that both in frogs and in mammals, after removal of these bodies, fatigue occurs very promptly on the performance of muscular work. The extract of the muscles of an animal dying as the result of removal of the bodies possesses a toxicity similar to that of the muscles of a normal animal tetanized to exhaustion. They infer that the muscular weakness following removal of the suprarenals is due to toxic substances of a similar nature to those producing physiologic

⁶⁴ Abelous and Langlois: *Arch. de Physiol.*, 1892, vol. iv, pp. 269 and 465.

⁶⁵ Albanese: *Arch. ital. de Biol.*, 1892, vol. xvii, p. 239.

fatigue, and that the function of the suprarenals is to supply antitoxic substances. In view of our present knowledge of the physiologic action of adrenalin in its various forms, it seems more probable that the weakness is to be explained by the absence of the normal tone-producing internal secretion of the bodies in question, and this is doubtless the true explanation of the muscular asthenia present in persons suffering from Addison's disease. Abelous, Charrin and Langlois⁶⁶ have published striking ergographic tracings of such cases.

Future research will probably reveal much regarding the relation between perverted metabolism and fatigue in pathologic conditions. In diabetes, for example, the possible fatiguing action of diacetic acid and of acetone ought to be investigated. In poisoning by phosphorus and by arsenic, in anemia and in certain diseases of the liver, sarcolactic acid is eliminated in the urine, and the physiologic action of this substance, already discussed, is probably responsible, in part at least, for the physical weakness accompanying these conditions. Because of the interest connected with the formation of aromatic bodies in the intestines as a result of putrefaction, and the supposed toxic action of some of these bodies on the central nervous system, at the suggestion of Professor Herter I have recently made a preliminary study of the action on muscle of indol, skatol and methyl mercaptan, and find all to possess some degree of fatiguing power.

In view of the fact that fatigue occupies so prominent a place in our daily life in both health and disease, it is strange that outside of the nostrum vendors not more serious endeavors have been made to provide specific antidotes for it. That various chemical substances delay fatigue is well known. I may be permitted here to refer to the study of the action of ethyl alcohol on muscle by Salant and myself⁶⁷ several years ago, in which we found that in medium quantity this substance exerts a favorable action, which is characterized by a quicken-

⁶⁶ Abelous, Charrin and Langlois: *Arch. de Physiol.*, 1892, vol. iv, p. 721.

⁶⁷ Lee and Salant: *Amer. Jour. of Phys.*, 1902, vol. viii, p. 61.

ing of the contraction; a quickening of the relaxation; the power of making a larger number of contractions and of performing a larger amount of work in a given time; an increase in the working time, or, in other words, a delay of fatigue; and the power of making a larger number of contractions and of doing a larger amount of work before exhaustion sets in. This action is exerted directly on the muscle protoplasm itself, not on the intramuscular nerve endings. In large quantity alcohol exerts an unfavorable action which is, in general, the reverse of that caused by medium quantities. The favorable action seems to be followed by unfavorable after-effects. Alcohol can not, therefore, be considered as worthy of ranking among the valuable antidotes to fatigue. And the same may be said of other substances of similar physiologic properties. A true antidote must recognize the causes. Both scientific experience and the experience of unscientific mankind seem to have demonstrated the real value of sugar in its various forms as a partial restorer of working power, and the old wives' prescription of cooking soda for one's tired feeling is certainly justified by the administration of sodium bicarbonate in advanced diabetes, if indeed this substance is not of actual benefit in ordinary daily physiologic fatigue. But at best sugar and alkali are only in part efficacious, and mankind at present can administer no food or drug that can push the wearied cells up the metabolic grade, either simultaneously with their descent or quickly after the descent has ceased. Only the assimilation and detoxication that normally come with rest—and, best, rest with sleep—are capable of adequate restoration of working power. Fatigue is a phenomenon of metabolism; recovery is a phenomenon of metabolism. To-day's research reveals only how much more complex is the body's metabolism than yesterday we thought it to be, and in the problem of fatigue there is probably much more before us than behind us.

THE FORMATION OF URIC ACID*

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THE rôle of uric acid in physiology and pathology has long formed a theme for unrestricted discussion and controversy in scientific literature. The wealth of published contributions on this subject abounds in the most characteristic specimens of what a recent medical writer has aptly termed "truth and poetry about uric acid."

Nevertheless the student of the physiology of uric acid can not fail to recognize a number of distinct epochs representing the introduction of new discoveries and consequent altered points of view in the interpretation of familiar phenomena. When the secret of each novel attitude is revealed it no longer becomes difficult to follow the march of progress in science; and, what is all-important, we are thus enabled to revise or formulate anew the problems with the solution of which advancement is coincident.

This Society, so happily inaugurated "for the diffusion of the knowledge of the medical sciences," will accomplish great good if its proceedings emphasize that before pathologic variations can be appreciated adequately, some fundamental knowledge of the normal functions of organs is essential. To the neglect of this, as a general rule, is due not a little of the confusion regarding the part played by uric acid in the animal body. Clinical observations, not infrequently confined to isolated cases or limited numbers, and more often carried out with inaccurate methods of research—the so-called clinical methods—have led to theories which were only reluctantly abandoned. Every investigator will admit that an hypothesis is a great aid to research in any domain; but a theory is admis-

* Lecture delivered February 10, 1906.

sible only so long as it fits the facts. Yet how difficult it is to relegate to the rubbish heap a theory which is supported by the authority of a distinguished worker. The present generation of scientists is learning to present truths eagerly, but to generalize from them with caution.

EARLIER VIEWS.

The obstacles to scientific progress which I have just referred to are well illustrated by some earlier views on the origin of uric acid in the body. Since urea was recognized as an end product of the oxidative decomposition of proteids nothing was easier than to assume that the larger molecule, uric acid, represents a product of incomplete oxidation. Whether uric acid was regarded as an intermediate product in the combustion of the large proteid complex or whether it was, rather, the outcome of an abnormal or irregular disintegration change—in either event it seemed proper to attribute an increased occurrence of the compound to a disturbance in the usual oxidation of ordinary proteids. In clinical language the vague uric-acid diathesis became the expression of an equally vague lowering of the oxidative capacity of the organism. It was observed, for example, that leukemia is often accompanied by an increased output of uric acid in the urine; and at once the theorists attempted to associate this with an insufficient oxygen-carrying capacity of the circulating blood, in connection with a relative diminution in the number of erythrocytes. The increasing number of cases reported failed to show any constancy in the increase of uric acid excreted. When finally the crucial physiologic experiment of interference with respiration was made, no increase of uric acid was noted; and, above all, when the actual conditions in disease were experimentally investigated, it was found that leukemia is not attended with any deficiency in oxidative powers. With these negative results the structure of the old theory of the incomplete oxidation of proteid fell. How much would have been gained in this instance alone by a more inductive method of study.

My subject is restricted to one aspect of the physiologic

problems concerning uric acid, namely, its formation in the body. How many erroneous ideas have arisen from a mistaken identification of the excretion of uric acid with its formation no one can say. So long as uric acid was looked on as one of the end products of nitrogenous catabolism the confusion was a natural one. We have, however, lately come to recognize this chemical compound as an intermediary product which may, in turn, experience ready decomposition within the body. With this once clearly recognized it is evident that the amount of uric acid eliminated within any given period is not necessarily any adequate index to that which has been formed within or introduced into the organism; and this statement applies independently of any undue retention to which attention has been directed so zealously by certain writers. The physiologist is to-day in a position to recognize the precursors of uric acid in the ingested foods or the disintegrating tissues and likewise to estimate with accuracy the quantities of these compounds which escape from the organism unchanged. And if the chemical and physiologic history of the intermediary transformations is not yet unfolded in its entirety, we can at least report commendable progress.

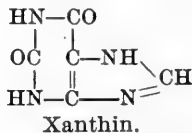
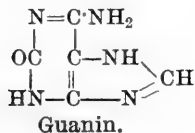
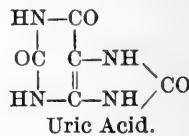
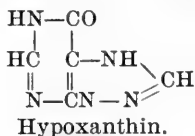
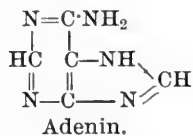
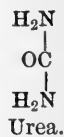
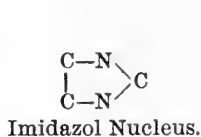
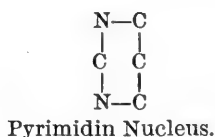
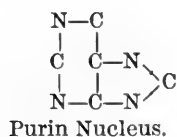
A review of early theories is unnecessary.¹ Manifold experimental investigations have long since demonstrated the untenability of the view ascribing the origin of uric acid to a decomposition of simple proteids, in which it might arise as an antecedent of urea. Similarly the notion of attributing the production of uric acid to a diminished oxidative capacity in the body as a whole may be dismissed, although we shall see that certain greatly modified aspects of this view have again arisen in other connections. The more adequate, or what

¹ For a comprehensive review of the older literature on uric acid in physiology and pathology, the reader is referred to the papers by Wiener: *Ergebnisse d. Physiol.*, 1902, vol. i, part 1, 1903, vol. ii, part 1; Burian and Schur: *Pflüger's Arch.*, 1900, vol. lxxx; 1901, vol. lxxxvii; Walker Hall: "The Purin Bodies of Foodstuffs," 1903. No attempt is made in the present paper to give a complete list of the newer investigations, only the more important references being noted.

may appropriately be termed the modern conception of the formation of uric acid is associated especially with the researches of two investigators, Kossel and E. Fischer.

THE WORK OF KOSSEL AND E. FISCHER.

E. Fischer definitely established the chemical structure of uric acid and a large group of related compounds which have become familiar, through their physiologic and pharmacologic bearings as the xanthin compounds, the alloxuric compounds or (more appropriately) the purin compounds. No physician can hope to have an adequate appreciation of the problems under discussion without some familiarity with the chemical structure of this group of related substances. The formulæ here presented therefore demand no apology; a superficial inspection alone reveals relationships which a page of description can not make equally evident.



Kossel's epoch-making contribution consisted primarily in his demonstration that the purin compounds, such as xanthin and hypoxanthin, are disintegration products of the widely distributed nucleoproteids, nucleins or nucleic acids. In one of his earliest papers, in 1881, recognizing the near chemical relation of the purin compounds to uric acid (see formulæ), he wrote: "The thought is at once suggested that these compounds play a significant rôle as normal precursors of uric acid or urea. The difficulty involved in the recognition of this lies

in the circumstances that these substances have heretofore been found in organs in small quantities only." Kossel² proceeded to show that the proportion of the different purin derivatives present in or obtainable from the body is far greater than had been anticipated.

The earliest experimental attempts which might have served to verify Kossel's hypothesis gave negative or uncertain results in almost every case. These failures are in part explicable in the light of subsequent study. A part of the difficulty is ascribable to inadequate analytical processes, a fact which is deserving of note at a time when the patient endeavors of the students of accurate analytical methods receive scanty appreciation from those who have no immediate use for them. Uric acid was at first missed as an end product of hypoxanthin-feeding, guanin-feeding and nuclein-feeding, partly owing to inadequate methods, in part probably because of poor absorption of the ingested materials, and more likely, further, because of the destruction of the formed uric acid in the intermediary metabolism of such species (dog, rabbit) as were inadvisedly selected for the trials.

HORBACZEWSKI'S EXPERIMENTS.

In 1889 Horbaczewski gave a new turn to the experiments and theories on uric-acid formation. To him was due the first experimental proof of the transformation of purin compounds into uric acid. By digesting organs like the spleen, rich in nucleoproteids, with blood, he obtained either the purin bases xanthin and hypoxanthin, or uric acid, according as his experiments were conducted in the absence or presence of oxygen. These experiments have become classic. But Horbaczewski made an equally important addition when he fed the nuclein prepared from spleen pulp and succeeded in eliciting an increased output of uric acid in both man and animals, thus transferring to the living body the experience gained in the digestion beaker.

Horbaczewski was unfortunate in the explanation which he

² Kossel: *Zeitft. f. physiol. Chemie*, 1881, vol. v, p. 267.

gave for this new production of uric acid. He attributed it to the disintegration of nucleoproteid material, it is true; but he persistently refused to credit the increase to the purin-yielding substances ingested. Accordingly, he invented the theory of a leucocytosis induced by the ingested nucleoproteids, leading in turn to a breaking down of body cells,—leucocytes rich in the purin-bearing nucleic-acid groups. That the introduction of nucleic acids, either as such or as proteid salts (nucleins, nucleoproteids) may lead to an increased elimination of uric acid has since been demonstrated for many compounds of both animal and vegetable origin and in a considerable number of animal species; but the evidence is to-day abundantly in favor of a direct transformation of the ingested material without the intermediation of leucocytes. An increase in the digestive leucocytosis under this condition of feeding is the unusual rather than the usual occurrence; and the experience which I have gathered with my co-workers indicates that a characteristic metabolism of the purins follows the most diverse modes of introduction of the mother substances into the organism. Parenteral paths are equally effective.

THE PURINS AND URIC ACID.

The failure of the earlier attempts to demonstrate in animals a conversion of ingested free purin bases, such as are obtained by the destruction of nucleic acids and nucleins, into uric acid, raised the question: Is some peculiar chemical complex or union like that of the nucleic acid molecule requisite for the conversion of the purins to uric acid in the body, or can the free purin bases be thus converted? For example, can guanin or adenin as such be transformed to uric acid, or is the characteristic oxidation dependent on the manner in which these purin bases are bound up in nucleic acid and such uric acid forming products as thymus gland? It is conceivable that hypoxanthin, for instance, might exert a leucotactic action such as Horbaczewski assumed for the nucleins; in that event the increased formation and destruction of leucocytes would presumably be accompanied by an increased elimination of lib-

erated phosphoric acid. Krüger and Schmid³ have made these questions superfluous by demonstrating that even in man the ingestion of hypoxanthin, xanthin, guanin or adenin is followed by an increase in the output of uric acid. They have made clear that this phenomenon is in no way the outcome of a leucotactic action of the free purin bases fed and that the phosphoric-acid metabolism of the body undergoes no noticeable alteration which would indicate any coincident destruction or synthesis of nucleic acid in the tissues.

With the genetic relationship between the purins of the food, whether in the form of the free hypoxanthin of meat extract or the nucleoproteids of glandular tissues like sweetbreads and liver, once established, it is instructive to consider the quantitative relations between these two factors in metabolism. To what extent do the purins introduced contribute to the increased production and elimination of uric acid? When a balance is struck between the intake of purins, either free or combined, and the increase in output of uric acid in the urine as a measure of its production from the ingesta, a deficit is uniformly detected. It might be assumed that the purins fed are in part eliminated unchanged; indeed, it is well recognized that the urine contains other purin derivatives than uric acid. The quantity of these is, however, not appreciably or significantly altered by purin feeding except under unusual circumstances. A few actual figures will serve to illustrate the deficit to which reference has been made. Thus, in one case in man after feeding 1.2 gm. of nitrogen in the form of hypoxanthin, 0.77 gm. was recovered as excess of uric acid, or about 62 per cent.; in the case of adenin 33 per cent. was recovered as uric acid, and less than 4 per cent. left the organism unchanged.⁴

DESTRUCTION OF URIC ACID.

The failure to recover the entire quantity of ingested purin in the form of uric acid or unchanged urinary purin suggests

³ Krüger and Schmid: *Zeitft. f. physiol. Chemie*, 1902, vol. xxxiv, p. 549. Earlier observations on hypoxanthin were reported by Minkowski and by Burian and Schur.

⁴ The data are taken from Krüger and Schmid: *Loc. cit.*, p. 559.

two explanations: 1, A retention of a portion of the purins or the uric acid formed therefrom so that they fail to reappear in the urine; or 2, a destruction of purins in the metabolic exchanges. The retention theory has been abandoned by almost all investigators except the followers of Haig, whose theories I regard as irreconcilable with modern experimental evidence. The question of the destruction of purins in the body, though foreign to the real subject of this lecture, nevertheless deserves brief consideration.

It has long been known that very considerable quantities of uric acid itself can be destroyed in the body. The evidence for this is made more convincing by the studies on various isolated organs in which this destruction has been investigated directly. For example, when blood containing a solution of uric acid is perfused through a surviving liver a progressive loss of uric acid takes place. The quantity of uric acid eliminated or found at any moment is therefore by no means an index to the quantity formed. The recognition of this fact, of the power of the animal organism to catabolize uric acid like other nitrogenous compounds, is one of the fruits of modern research which has profoundly changed our attitude toward the problems of purin metabolism.

The extent to which the destruction of uric acid and other purins actually takes place varies in different animal species. Burian and Schur⁵ have made an elaborate study of this subject and reached the conclusion that the proportion of the ingested purins which escapes destruction and is therefore eliminated as uric acid, is rather constant. In carnivora, like the dog and cat, it is about $1/20$; in herbivora, like the rabbit, $1/6$, and in man $1/2$ of the purin material introduced. This explains why so little uric acid is ordinarily found in the urine of the familiar laboratory animal, the dog, in contrast with man. It is furthermore probable that young animals are not equally capable of effecting the destruction of uric acid,

⁵ Burian and Schur: Pflüger's Arch., 1901, vol. lxxxvii, p. 335. Cf. also Krüger and Schmid: Loc. cit.

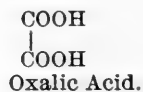
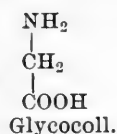
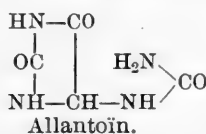
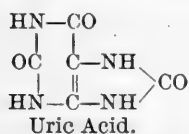
and it is not unlikely to me that similar defects may be associated with pathologic conditions.

If we summarize these recent findings respecting the destructive capacity of the body for uric acid, a new point of view arises in the discussions of the so-called uric-acid diathesis. Uric acid formed in the body is ordinarily largely destroyed in the body again; it may therefore occur in unusual amount owing to diminished destruction equally as well as it might accumulate through imperfect elimination or increased production. The important point is that a study of the urine alone will not decide. The tolerance of the organism for purins must be ascertained by a comparison of the intake.

What are the end products of this destruction of uric acid in the organism? Urea is at once suggested by the mere inspection of the structural formulæ. The possible formation of oxalic acid



in the same reaction is also thus brought into view. In the carnivorous dog and cat the observations of Salkowski, Minowski, Cohn and the work in our laboratory, have indicated that allantoin represents an important transformation product of uric acid and the purins. A further decomposition product is glycocoll. The chemical relationships involved are made clear by the comparison of the structural formulæ:



The current views regarding the relative formation of these compounds under different circumstances are still divergent and a discussion of them at this time would be unprofitable.

The review which has been presented up to this point has traversed the paths familiar to every student of this subject. At most the changing ideas regarding the origin of uric acid have been pointed out. The distinctly modern aspect of the problem concerns the mechanism by which the metabolism of the purins

is effected—the chemical history of the reactions which yield uric acid from nucleoproteids, and in turn cause its disappearance.

ENZYMES AND PURIN METABOLISM.

That a preponderating rôle is played by soluble enzymes is no longer a matter of conjecture; nor is this anything other than what the increasing knowledge of the importance of these biologic agents would lead us to anticipate. Enzymes are no longer thought of exclusively as agents of the digestive apparatus; they enter everywhere into the manifold activities of cells in almost every feature of metabolism. Hydrolysis and oxidation are facilitated by their participation in definite, conceivable ways. Horbaczewski's⁶ observations on the formation of uric acid *in vitro* from the purins of spleen pulp formed the starting point for the new departure. Spitzer⁷ then demonstrated that extracts of spleen and liver may yield uric acid when air is present, even in the absence of putrefactive processes, thus indicating the enzymatic character of this reaction. He also showed that uric acid can be obtained in an oxidative way from adenin and guanin by the action of some constituent of spleen or liver extracts in the presence of atmospheric oxygen, although the formation of uric acid is far more extensive when xanthin and hypoxanthin are the precursors. These results were confirmed by Wiener,⁸ and the facts which they represent are now undisputed.

The additional contributions of Schittenhelm⁹ and Jones¹⁰ have established the co-operation of several enzymes in the steps leading to uric acid as an end product. The first of these involves a nuclease, that is, an enzyme capable of splitting

⁶ Horbaczewski: *Monatsh. f. Chemie*, 1891, vol. xii, p. 221.

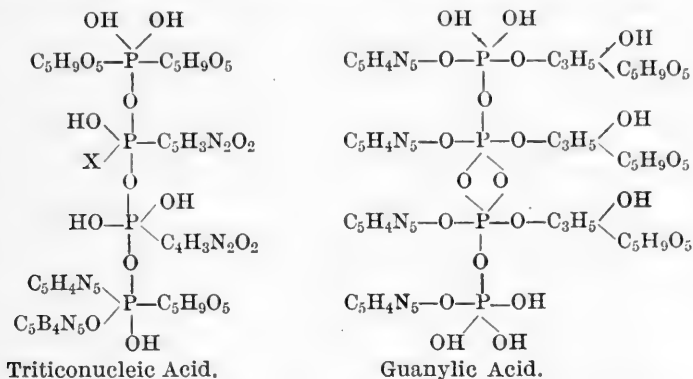
⁷ Spitzer: *Pflüger's Arch.*, 1899, vol. lxxvi, p. 192.

⁸ Wiener: *Arch. f. exper. Pathol. u. Pharmak.*, 1899, vol. xlii, p. 373.

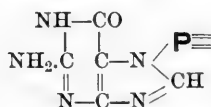
⁹ Schittenhelm: *Zeitft. f. physiol. Chemie*, 1904, vol. xlii, p. 251; vol. xliii, p. 228; 1905, vol. xlv, p. 121; vol. xlvi, p. 354.

¹⁰ Jones: *Ibid.*, 1905, vol. xlv, p. 84, and earlier papers therein mentioned.

up nucleic acid so as to liberate the purin bases present in its complexes. The constitution of the nucleic acids is by no means determined, although the researches of Osborne¹¹ and of Bang¹² enable one to obtain some conception of the possible relationship of the purin derivatives to the remaining radicals, as indicated in the provisional formulæ:



Burian¹³ has lately given reasonable evidence that the purin groups are attached to the remaining nucleus at the 7-position of the purin skeleton, so that guanine, for example, would be attached to the remaining groups as indicated below:



In chemical terms the action of the enzyme nuclease would thus be comparable with the reaction involved in the hydrolysis of an acid amide:



Nucleases are doubtless widely distributed in nature. They must obviously share in many autolytic processes in organs and tissues. At present, however, our information regarding this class of enzymes is rather limited.

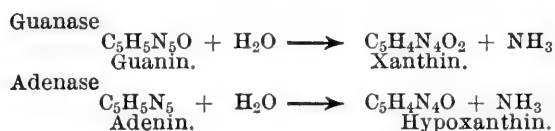
¹¹ Osborne and Harris: *Ibid.*, 1902, vol. xxxvi, p. 85.

¹² Bang: *Ibid.*, 1901, vol. xxxi, p. 411.

¹³ Burian: *Ergebnisse d. Physiol.*, 1904, vol. iii, 1, p. 90; Johnson: *Amer. Chem. Jour.*, 1905, vol. xxxiv, p. 197.

More light has been thrown on a second step in the intermediary metabolism of purins. Several investigators, notably Jones¹⁰ and Schittenhelm,⁹ have succeeded in isolating enzymes of the amidase type which accomplish the conversion of the amino-purins, guanin and adenin, into xanthin and hypoxanthin, with liberation of ammonia.

Reactions of this kind, attributable to enzyme influence, have long been recognized and assumed to have importance in the deamidizing of nitrogenous compounds in metabolism.¹⁴ For the cases under consideration the reaction may be assumed to proceed as follows:



In correspondence with this, adenin and guanin are converted into hypoxanthin and xanthin, respectively, when they are digested with extracts of certain tissues. An interesting controversy has arisen in the study and developed into more than polemical interest. Having obtained extracts of organs which readily deamidize adenin without converting guanin, Jones very properly postulated the existence of two distinct amidases—guanase and adenase. Working with the same organ (the spleen), Schittenhelm has failed to note any such distinctive difference. The discrepancy has lately been cleared up by the discovery of Jones,¹⁰ that whereas the spleen of the ox (with which Schittenhelm experimented) contains guanase, it is missing in that of the pig (which formed the source of Jones's experimental material); and the latest experiences of Schittenhelm¹⁵ and of Pfeiffer¹⁶ add to the growing conviction that the distribution of the enzymes concerned in the metabolism of the purins is very unlike in different species. Future

¹⁴ Cf. Lang: Hofmeister's Beitr. z. chem. Physiol., 1904, vol. v, p. 321.

¹⁵ Schittenhelm: Zeitft. f. physiol. Chemie, 1905, vol. xlvii, p. 354.

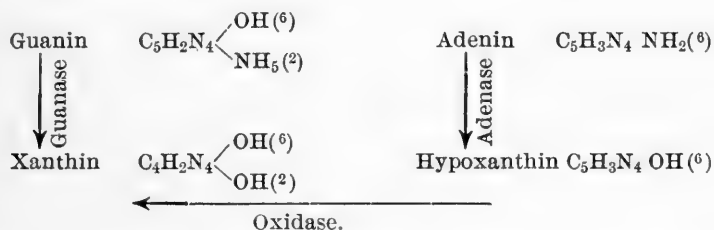
¹⁶ Pfeiffer: Hofmeister's Beitr. z. chem. Physiol., 1905, vol. vii, p. 463.

studies in this field will doubtless contribute many features of interest in comparative physiologic chemistry.

That these enzymes are not universally distributed in all organs and tissues is certainly not without physiologic significance. Up to the present adenase and guanase have been found in the spleen, liver, lung, muscle, thymus and suprarenals; not in the intestines, blood or kidneys.¹⁷ When tissue extracts prepared from spleen, liver, muscle or lung are allowed to act on guanin or adenin in the presence of oxygen, uric acid is found in place of xanthin or hypoxanthin. In this reaction a third enzyme, an oxidase, is involved, converting hypoxanthin to xanthin and subsequently to uric acid.



Under favorable conditions the conversion may be practically complete. The first step in these reactions may be graphically represented as follows:¹⁸



The final step is the oxidative transformation of xanthin into uric acid. I am unable in the allotted time to give appropriate recognition to the various investigators who have at length enabled us to trace in some comprehensible way the intermediary changes by which the purins and their nuclein precursors may give rise to uric acid; nor am I permitted to do more than refer to the work of Wiener, Ascoli, Schittenhelm and others,¹⁹ in which the destruction of uric acid by the agency of uricolytic enzymes has been found conspicuous in the kidney and liver, and in lesser degree in extracts of muscle

¹⁷ Schittenhelm: *Zeitft. f. physiol. Chemie*, 1904, vol. xliii, p. 236.

¹⁸ Jones: *Ibid.*, 1905, vol. xlv, p. 2.

¹⁹ Schittenhelm: *Ibid.*, 1905, vol. xlv, pp. 147, 161; *Almagia: Hofmeister's Beitr. z. chem. Physiol.*, 1905, vol. vii, p. 459.

and bone marrow. The sequence of events in the transformation of purins is bound up in a series of typical enzyme reactions.

THEORIES OF URIC-ACID SYNTHESIS.

Although most physiologists have adopted the theory of the formation of uric acid by an oxidative transformation of purins arising in intermediary metabolism, there is another possible mode of origin which can not be dismissed without due consideration. I refer to the direct synthesis of uric acid. That nucleic acid can be synthesized and the construction of the purin nucleus become possible in mammals is beyond question in the case of developing and growing animals living on a practically purin-free dietary.²⁰ That uric acid itself is formed by synthetic processes in birds is also verified beyond doubt. In mammals the end product of nitrogenous exchange is quite different, however. When the synthesis of uric acid had been accomplished in the laboratory repeated attempts were made to induce an analogous formation in animals. Horbac-

zewski's artificial synthesis from urea $\begin{array}{c} \text{NH}_2 \\ | \\ \text{CO} \\ | \\ \text{NH}_2 \end{array}$ and glycocoll $\begin{array}{c} \text{NH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{COOH} \end{array}$ could not be imitated by feeding these compounds; and similar failure in man followed the administration of ammonium sarco-

lactate $\begin{array}{c} \text{OH} \\ | \\ \text{C}_2\text{H}_4 \\ | \\ \text{COONH}_4 \end{array}$ and urea which Minkowski found effective in producing uric-acid synthesis in birds.

It has been noted in a few cases that the ingestion of considerable quantities of non-nitrogenous foods like fats or sugars may be accompanied by an increased elimination of uric acid. To what extent the conditions for the elimination of uric acid have been modified under such circumstances can not be decided; at most the data admit of a twofold interpretation. The most successful advocate of the immediate synthesis of uric

²⁰ Cf. Miescher: Arch. f. exper. Path. u. Pharmak., vol. xxxvii, p. 100; Kossel: Zeitft. f. physiol. Chemie, 1885, vol. x, p. 248; Burian and Schur: Ibid., 1897, vol. xxxiii, p. 55.

by certain decomposition fragments like the thymine, uracil and cytosine of nucleic acids) and uric acid; and from another aspect Knoop and Windhaus,²⁴ having obtained methyl imidazole by the action of ammonia on sugar, point out the relation of this compound to the purin nucleus. Such speculations, devoid of all experimental support, are at present of interest only in pointing out the possible roads to a synthesis of uric acid within the body.

Unmindful of the direct evidence of purin oxidation afforded by the enzyme experiments with tissue extracts and emphasizing the difficulty of obtaining uric acid directly from purins in the laboratory without the intervention of animal factors, Kutscher and Seemann²⁵ have ventured another explanation according to which uric acid is the primary product, formed by such synthetic reactions as have just been detailed. A part is in turn reduced, according to Kutscher and Seemann, to simpler purins which participate in the genesis of nucleins in the body; the excess of synthesized uric acid is then either eliminated or destroyed in the usual manner. On this hypothesis the feeding of purins would give rise to increased uric acid elimination by sparing the synthetic uric acid from reduction, etc., to form nucleic acid and thus inducing a certain excess of it in the system. Experimental evidence of such reduction changes of uric acid to simpler purins is entirely wanting. The capacity of the organism to oxidize purin bases to uric acid, as evidenced by feeding experiments and trials with tissue extracts, remains as the single indisputable fact advanced beyond the realm of hypothesis.

ENDOGENOUS AND EXOGENOUS PURINS.

On a diet free from nucleoproteids and purins, as well as in starvation, the elimination of uric acid and other purin bases does not cease by any means. Under such conditions some

²⁴ Knoop and Windhaus: Hofmeister's Beitr. z. chem. Physiol., 1905, vol. vi, p. 392.

²⁵ Kutscher and Seemann: Centrbl. f. Physiol., 1903, vol. xvii, p. 715.

metabolic change like the destruction of tissue nucleoproteid must supply the purin nucleus for the eliminated product.

Burian and Schur²⁶ have designated that fraction of the purin output which is independent of ingested purin groups as endogenous in contrast with the exogenous elimination referable to preformed purin complexes in the intake. They note that on a purin-free diet consisting of such foods as milk, eggs, cheese, potato, rice, green vegetables, wheat bread, butter and sugar, the endogenous uric-acid output is a rather constant quantity for any individual under fixed conditions of living and is practically independent of the total proteid or energy content of the diet. With this the observations of Sivéⁿ²⁷ are in accord. The constants of endogenous purin output vary considerably for different individuals, however, as is shown by additional studies of my co-workers, especially Rockwood,²⁸ and by Kaufmann and Mohr.²⁹ This conception of the different sources of the uric acid eliminated and the determinable endogenous constancy of the individual has been helpful in the study of purin metabolism in both the laboratory and the clinic. For it has enabled the tolerance, or degree of utilization and elimination of purins to be determined with quantitative precision, by estimation of the exogenous uric acid derived from diets of known composition.

Recent experiments by Folin³⁰ and von Wendt,³¹ as well as unpublished observations of my own,³² indicate that the final word regarding the endogenous purin metabolism has not been spoken. When the total amount of protein metabolism is greatly reduced the endogenous output of uric acid is diminished, though this is not the case within ordinary ranges of diet.

²⁶ Burian and Schur: Pflüger's Arch., 1900, vol. lxxx, p. 269.

²⁷ Sivéⁿ: Skandinavisches Arch. f. Physiol., 1901, vol. xi, p. 123.

²⁸ Rockwood: Amer. Jour. of Physiol., 1904, vol. xii, p. 38.

²⁹ Kaufmann and Mohr: Deutsches Arch. f. klin. Med., 1902, vol. lxxiv, pp. 141, 348.

³⁰ Folin: Amer. Jour. of Physiol., 1905, vol. xiii, p. 86.

³¹ von Wendt: Skandinav. Arch. f. Physiol., 1905, vol. xvii.

³² On the output of uric acid in man during prolonged starvation.

URIC ACID AND MUSCULAR METABOLISM.

Some explanation of these discrepancies may be expected in the near future, especially since Burian³³ has lately identified in the muscle purins, notably hypoxanthin, the source of endogenous purins eliminated. He has found that during activity the purin-content of a surviving muscle is increased, and that in the perfusion of muscular parts the circulating fluid carries away uric acid from the muscle both in rest and during contraction. The effective factors thus are the continual accumulation of purins (hypoxanthin) in the muscles, an enzymatic production of uric acid therefrom by the localized xanthin oxidase of the muscle cells and its continuous removal by the blood current. By work the output of uric acid is temporarily increased, though it becomes equalized again in the course of the usual 24-hour period so that an effect of muscular activity on uric acid elimination measured in the daily output has generally been overlooked.

As a tentative hypothesis the conclusion of Burian may be quoted: "From a quantitative point of view the participation of the metabolism of muscles in the formation of endogenous urinary purins in man is probably large. This is made evident, for example, by the fact that the (hourly) endogenous purin output during a condition of muscle fatigue, *i.e.*, presumably diminished hypoxanthin formation in muscle, is sometimes reduced one-half. Obviously only a very small portion of the endogenous urinary purins is derived from the nucleoproteids of disintegrated cells, whereas a very considerable part arises from muscular metabolism. This mode of origin also well explains the behavior of the 24-hour endogenous urinary purin elimination, namely, that it is constant in a single individual even after marked variations in the food intake, provided that the habits of life are reasonably uniform; whereas in different individuals, especially if their musculature is of unlike development, the output may be quite unlike."³⁴

³³ Burian: *Zeitft. f. physiol. Chemie*, 1905, vol. xliii, p. 532.

³⁴ Burian: *Loc. cit.*, pp. 544 to 545.

Herein may lie the explanation of the differences in hourly output of endogenous uric acid in the day time and at night. In the latter case, during muscular rest, it is always lower, as shown in the following data by Rockwood from a subject living on a fixed purin-free diet. The constancy of the total daily uric output is likewise evident.

THE ELIMINATION OF ENDOGENOUS URIC ACID.

The daily diet consisted of :

Milk	1,350 c.c.
" Force "	35 gms.
Cream	50 gms.
Sugar	20 gms.
Oyster crackers	250 gms.
Cheese	30 gms.
Eggs	96 gms.
Apple	90 gms.
Wheat bread	25 gms.
Butter	15 gms.
Estimated fuel value	2,770 calories.

COMPOSITION OF THE URINE.

Day.	Day Urine, per Hour.			Night Urine, per Hour.			Total Urine.		
	Nitro- gen. gm.	Uric Acid. gm.	P ₂ O ₅ . gm.	Nitrogen. gm.	Uric Acid. gm.	P ₂ O ₅ . gm.	Nitro- gen. gm.	Uric Acid. gm.	P ₂ O ₅ . gm.
1	0.485	0.0138	0.092	0.512	0.0106	0.090	11.87	0.303	2.20
2	0.572	0.0161	0.108	0.458	0.0089	0.085	12.70	0.321	2.39
3	0.521	0.0135	0.109	0.532	0.0095	0.106	12.60	0.289	2.59
4	0.535	0.0148	0.110	0.499	0.0104	0.089	12.48	0.311	2.42
5	0.543	0.0143	0.115	0.564	0.0124	0.090	12.23	0.325	2.63
6	0.540	0.0145	0.111	0.530	0.0117	0.102	12.87	0.323	2.57
7	0.546	0.0156	0.109	0.530	0.0113	0.098	12.96	0.336	2.52
8	0.537	0.0142	0.111	0.519	0.0090	0.101	12.72	0.294	2.57
Daily average.	0.535	0.0146	0.108	0.518	0.0105	0.095	12.68	0.313	2.49

SEAT OF URIC ACID FORMATION.

Next in importance to an adequate appreciation of the chemical process by which uric acid originates in metabolism is the recognition of the place where its formation occurs in the organism. Various considerations have been brought to bear on the solution of this question, complicated as it is now known to be by the occurrence of formative and destructive changes simultaneously. Some of the older modes of attacking the problems are readily seen to be fallacious. For example, the

fact of the relative abundance of uric acid in particular organs can not properly be explained by any immediate formation of the compound in those tissues, since the result noted may merely be the expression of a temporary deposition in the organ.

The spleen is still regarded in many text-books as an important factor in the production of uric acid, doubtless owing to the original observation by Horbaczewski on the occurrence of this substance in spleen pulp. The observations of Jackson and myself³⁵ on splenectomized animals, and of Gibson and myself³⁶ on a splenectomized man, have shown that the spleen by no means plays a preëminent rôle in purin metabolism, since both exogenous and endogenous uric acid excretion proceeds undiminished in the absence of that organ. In birds the importance of the liver for the synthesis of uric acid has been exhibited beyond question; but it does not assume an equally significant position in mammals, for a considerable excretion of uric acid continues in animals in which the liver is excluded from the circulation by an Eck fistula.

In the classic observations of Hahn, Massen, Nencki and Pawlow³⁷ the output of uric acid was, if anything, found increased after exclusion of the liver—a result corresponding with my own unpublished experiments on various pathologic manifestations of the liver as obtained in the clinic or experimentally induced in animals.³⁸

Experience of this character has long brought the conviction to me that uric acid is formed in various parts of the body—the muscular tissue being prominently suggested—and that the exceptionally large quantities eliminated when the hepatic functions are impaired may be the expression of a deficient destruction of the uric acid formed throughout the body in

³⁵ Mendel and Jackson: *Amer Jour. of Physiol.*, 1900, vol. iv.

³⁶ Mendel and Gibson: *Ibid.*, 1904, vol. x, p. xxix.

³⁷ Hahn, Massen, Nencki and Pawlow: *Arch. f. experimentelle Path. u. Pharmak.*, 1892, vol. xxxii, p. 161; also M. Nencki: "Opera Omnia," vol. ii, p. 314.

³⁸ Cf. Mendel and Jackson: *Loc. cit.*, Table IV.

intermediary metabolism and ordinarily in large part further disintegrated in the liver. The most satisfactory further evidence of the probable unlocalized genesis of uric acid in mammals is afforded by the significant widespread distribution of the enzymes affecting purin metabolism.

Summarizing ³⁹ once more the newer observations it appears that especially the spleen, lungs, liver, intestine, muscle and kidney (in some animals at least), are all capable of converting purin bases into uric acid; and that the kidney, muscle and liver can, in turn, further disintegrate the newly formed uric acid—a reaction not accomplished by the spleen or lung. Guanin is first converted into xanthin, which is then oxidized to uric acid; while adenin is first transformed into hypoxanthin from which xanthin subsequently arises. An abundant supply of oxygen is essential in every case for the change to uric acid and the catalog of effective or inactive organs is presumably not yet complete. Uric acid formation and destruction by no means always proceed together. The uricolytic or destructive ferment is doubtless principally confined to a few distant organs, especially the kidney and liver. Differences in the distribution of these active agents unquestionably occur in different species; and the possibility of an elimination, acceleration or retardation of these enzymatic processes by disease or the action of drugs suggests many new problems. At any rate, the newer chemical studies have at length greatly enlarged the horizon of this field of investigation.

RATE OF URIC ACID FORMATION.

The only approach to determining the rate of uric acid formation in man at present consists in observing the rate of elimination—a method the uncertain significance of which has already been pointed out. Nevertheless, bearing in mind the indefiniteness of the conclusions afforded, it is interesting to note that the curve of endogenous uric acid output from hour to

³⁹ In what follows I have referred freely to the recently published résumé of the researches of Schittenhelm: *Zeitft. f. physiol. Chemie*, 1905, vol. xlv, p. 146.

hour tends to attain a level with some slight rise, perhaps, in the morning hours. This has been shown by Dr. E. W. Brown and myself in unpublished experiments, by Pfeil,⁴⁰ by Sootbeer,⁴¹ and by Beebe.⁴²

After purin-containing meals an increased hourly output of uric acid is induced in a comparatively short period, giving rise to a typical curve for the individual. Sootbeer has made the interesting observation that in arthritis urica, the typical curve of elimination after purin-containing meals (meat) may show great irregularity in contrast with that obtained from healthy persons. The typical postprandial rise in uric acid output may, for example, be delayed or missed altogether.

These facts suggest a new method of study applicable to some of the problems of the uric acid diathesis. The influence of drugs like alcohol is brought out in characteristic form by a study of the hourly elimination of uric acid. When no meal is taken even large doses of alcohol fail to produce a marked effect on the hourly elimination, despite the diuresis which they incite. This is well shown in the following table from Beebe:⁴³

Hour.	Urine Volume. c.c.	Uric Acid. mgm.
7-8	57	30.4
8-9	84	33.0
9-10	110	31.5
10-11*	465	26.5
11-12	565	29.2
12-1	57	27.8
1-2	35	28.8
2-3	30	24.0
3-4	20	20.8

* Alcohol taken at 10 a. m.

The postprandial rise in uric acid elimination is, however, greatly exaggerated by ingestion of alcohol, especially when a meal rich in purin constituents is taken. The contrast is

⁴⁰ Pfeil: Zeitft. f. physiol. Chemie, 1903, vol. xl, p. 1.

⁴¹ Sootbeer: Ibid., p. 25.

⁴² Beebe: Amer. Jour. of Physiol., 1904, vol. xii, p. 13.

⁴³ Beebe: Loc. cit., p. 20.

seen in the following averages compiled from many comparable results by Beebe:⁴⁴

Control Day.		Alcohol Day.
Hour.	Uric Acid. mgm.	Uric Acid. mgm.
9	18.9	19.0
10	15.7	16.3
11	17.1	16.4
12*	16.1	14.4
1	12.8	16.2
2	15.6	19.5
3	19.3	25.7
4	23.6	28.3
5	24.8	34.2
6	25.5	28.8

Residue of Twenty-four Hours.

Control Day.	Alcohol Day.
Uric Acid 338 mgm.	Uric Acid 442 mgm.

*Meal hour.

Evidently the purin metabolism is noticeably modified by the alcohol absorbed. Bearing in mind the two-fold action—uric acid formation and destruction—which the tissues are capable of, Beebe has interpreted his results to indicate an inhibition of the destructive (uricolytic) phase. The fragmentary data regarding the rate of uric acid elimination have been briefly referred to in this place in order to indicate some of the newer procedures which are being brought to bear on the production of uric acid in health and disease. They contribute little new to the problems with which we have been more directly concerned.

FUTURE PROBLEMS.

Thus I have attempted to subject to a somewhat critical review the more recent experimental observations which should contribute to a better understanding of the formation of uric acid in the body. Its probable endogenous and exogenous antecedents have been considered and the probable chemical

⁴⁴ Beebe: Loc. cit., p. 30.

reactions involved in the genesis of uric acid from other purin compounds have been detailed. Attention has also been directed to the peculiar rôle of enzymes in both the formation and destruction of uric acid; the organs and tissues concerned, as well as the factors modifying these metabolic changes, have been considered. After all, a résumé serves its best purpose in enabling the scientist to appreciate the limitations of his methods and to learn the true worth of his data, so that from time to time he may formulate his problems more precisely. He thus starts anew in his quest of the truth with better defined aims and a consequent renewal of enthusiasm.

Research brings forth questions as well as answers. The patient reader will have discovered many gaps in the chain of uric acid metabolism, where the missing links remain to be discovered. We assuredly need to know more about the origin and significance of endogenous uric acid. How and why is it modified in such physiologic states as starvation, in growth and in disease—in conditions where “the metabolic processes that determine the uric acid excretion may be said to be in a relatively unstable equilibrium?” What interpretation shall be given to variations in the endogenous output; and what is the tolerance of the body for purins in diverse conditions? What determines the balance between formation and destruction of uric acid and how do the different tissues participate in its chemical regulation? Are the differences in the purin metabolism of unlike species explicable by qualitative or quantitative considerations? These and many other inquiries at once present themselves; and the patent limitations of our knowledge stimulate the investigator to renewed efforts and awaken his interest.

THE EXTENT AND LIMITATIONS OF THE POWER TO REGENERATE IN MAN AND OTHER VERTEBRATES*

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ABOUT the middle of the eighteenth century great interest was aroused in the power shown by certain animals to replace lost parts. The remarkable work of Trembley, Bonnet and Spallanzani made known some of the principal results with which we are familiar to-day. Many new facts have also been discovered since their time, but despite the fundamental importance of the phenomena of regeneration, little is known at present in regard to the physiology of the process. Nevertheless, a beginning in the study of the physiology of regeneration has been made, and I invite your attention especially to some of the more general aspects of this side of the problem.

In this connection I should like to discuss also the question why certain animals seem to lack the power to replace lost parts; and since man himself belongs to this class, the meaning of the fact is of direct and, perhaps, even of practical importance to us; for if we could determine why man does not replace a lost arm or a leg, we might possibly go further and discover how such a process could be induced by artificial means.

It has seemed to me that regeneration is only one phase of the general phenomenon of growth. If this is the case why does an animal that has ceased to grow begin to regenerate with great rapidity when a part is removed? If we turn the question around the other way and ask, why does an animal stop growing when a certain size is reached, we may attack the problem at closer quarters.

* Lecture delivered February 17, 1906.

WHY DOES AN ANIMAL STOP GROWING?

In the first place, an animal stops growing not because its cells have lost their power of further growth. This much seems certain; for, if a part is removed all the cells at the cut surface begin to grow again. Moreover, this new growth does not take place as some writers have assumed from reserve cells, but from the formed tissues of the old parts. We must conclude, therefore, that in many, perhaps in all animals, the cells, with possibly few exceptions, still possess the power of further growth. In fact the cells seem to have limitless powers in this direction, and something in the body must restrain their activity after a certain size has been reached. What is the nature of this restraint? What retards the development as the size approaches that normal for the animal?

It has sometimes been assumed that growth is retarded or stopped because the animal can digest only so much food, and that the adult size is the stage of equilibrium between the amount of food digested and the amount of food used up. Now if this assumption is true, we might attempt to test it in the following way:—If we remove a part of an animal, a tail or a leg for example, the remainder of the body ought to grow larger, because the food that went to nourish the part removed can now be utilized by the rest of the body. Suppose, for example, that we cut off the tail of a salamander, we should expect, if this view is correct, that the animal would increase in weight by just as much as the weight of the tail removed. As the new tail grows out the whole weight should remain constant; for, what is added to the new tail, day by day, must be lost by the rest of the body. In other words, after the operation the weight should increase rapidly to what it had been before, and then hold its own as the new tail develops.

I have recently carried out an experiment of this kind. The results are given in the accompanying table. Two sets of salamanders were weighed; in one set the tails were amputated and the animals were again weighed. The other set was kept intact as a control. Both sets lived under the same conditions,

and were fed exactly the same amount of food. This is possible in the species that I have used, because the animals can be fed by hand and the quantity of food thus regulated. As the table shows, the amount of food given at first did not cause an

TABLE SHOWING GAIN IN DIMYCTYLUS WITH TAILS CUT OFF AT BASE
AND TAILS NOT CUT OFF.

With Tails		
1.48.....	Dec. 12.....	
Without Tails		With Tails
1.23.....	Dec. 12.....	1.85
1.36.....	Dec. 13.....	
1.49.....	Dec. 18.....	1.70
1.53.....	Dec. 27.....	1.66
1.83.....	Jan. 5.....	1.98
1.90.....	Jan. 13.....	2.06
1.91.....	Jan. 20.....	2.13
2.07.....	Jan. 27.....	2.37
2.02.....	Feb. 3.....	2.19
2.13.....	Feb. 10.....	2.23

increase in weight in the control set, but the tailless animals increased in weight and in a week had made good the weight lost in the tails. They also continued to gain weight more rapidly than the control set, which is perhaps a suspicious circumstance.

It should be noted that the animals were in an underfed condition when the experiment began, and the difference in the rate of increase in the two cases may not represent what an animal might do that had already reached its maximum size. I have repeated the experiment on the same salamanders in a well-fed condition and have not always found the same difference noted in the preceding case. Since these animals have no definite upper limit of growth, they proved to be not well suited to test the question, although the great initial increase in the tailless set may, in part, be due to the absence of the tail. The most interesting outcome of the experiment was that the increase in size is not due to the storage of fat, but to growth in all of the organs of the body. It might be concluded that the regeneration of a new part is due to the temporary increase of available food, owing to the loss of the old part, but that this

is not the explanation is shown by the following experiment:— If we amputate the tails in two sets of animals, and starve one set and feed the other, we find that the rate of growth of the new tail is nearly the same in the two sets. After two months the starved animals are greatly emaciated, but the length of their new tails is *almost* as great as that of the well-nourished individuals.

In other words, the new part grows at nearly the normal rate while the rest of the animal is starving to death. Clearly, then, the power of regeneration is not determined by the amount of food digested. It is due rather to the greater assimilative power of the cells of the new part.

In this connection another curious fact should be mentioned. It has recently been shown by Zeleny, for the crayfish and for brittle-stars, that the greater the number of legs or of arms removed the faster each one grows. If one leg is removed it regenerates at a given rate; if two are removed each regenerates faster than when only one is absent, etc. This result recalls Pflüger's celebrated teleologic law: that in living beings the cause of every need is at the same time the cause of the fulfillment of the need. For example, lack of food causes starvation, and starvation is the cause of the appetite that leads the animal to search for food.

I do not care to advocate this view, but simply to call attention to the neatness with which it covers the case of regeneration just given. The scientific explanation must be sought in some other direction. Zeleny has discussed the question whether his results may not be due to the amount of food. A certain amount of material is required to nourish a leg. If we remove the leg a surplus is present. If we remove two legs so much more surplus is present, which can go to nourish both new legs, and for a time both may grow faster than when only one leg is removed. If this were the real explanation of the increased rate of growth when more legs are removed it would follow that a starved animal would replace the two legs more slowly than a well-fed animal would replace one leg. I tested this possibility. One, two or three legs were removed from my

salamanders. One set was starved, the other fed, and the individuals in the two sets compared. Little or no difference between the two sets could be detected; hence, I conclude that the result is not primarily one of food supply.

THE RATE OF REGENERATION AT DIFFERENT LEVELS.

Perhaps the most important facts bearing on the problem under discussion are those connected with the rate of regeneration at different levels. If the tail of a fish is cut off near the base the new part grows faster than when the tail is cut off near its outer end. The new tail may be replaced as soon when much is cut off as when less is cut off. The result is independent of food, for it takes place in the same way whether the fish is starved or fed. This relation between the rate of growth and the amount removed is found to occur in widely different groups of animals. King has shown in the starfish that when an arm is cut off near the base the new arm regenerates faster than when the arm is cut off near its tip. In the earthworm I obtained similar results, for the posterior end at least. If a few segments are cut off from the posterior end they regenerate with extreme slowness. If the worm is cut in two in the middle a very large number of segments regenerates; if the worm is cut in two further forward, behind the girdle, a still larger number of segments is regenerated in the same time.

What is the cause of this difference in the rate of regeneration at different levels? I am not certain that I can answer this question, but I should like to call attention to a remarkable agreement between this result and the normal process of growth. Growth is much greater in youth than in adolescence, and becomes less and less as the adult size is approached. We find this same relation in the newly-regenerated part. It grows less rapidly the nearer the cut surface is to the complete form. It seems, therefore, not improbable that whatever regulates the rate of growth of the animal as a whole also regulates the growth of the regenerating part.

Some writers believe that regulative processes in general are

due to a vitalistic principle resident in living matter. In fact, the whole regenerative process has sometimes been referred to a mysterious formative or completing force, but so long as we do not know anything about such a force we gain nothing and lose a great deal, I think, by referring the process to such an intangible idea.

There is at least one physical possibility which in some form or other may be involved in the regulation of the growth process. Our problem, you will observe, seems to have narrowed itself down to determining an inhibitory factor, since we have assumed that the cells of the body possess unlimited possibilities of growth if given a suitable environment. It seems to me not improbable that the inhibition is caused by a definite response to a condition of mutual pressure or tension of the cells on each other. When this condition is reached further growth comes to an end. When we alter this particular pressure by removing a part, growth begins again.

What the nature of this pressure may be I can not say, nor how the cells respond to it, but taking all the facts into account, this assumption may at least give us an escape from the assumption of a formative force. Indeed, the formative force itself is nothing more, if my view is correct, than the response of the cells to the pressure relations of neighboring cells.

I am aware that this view is purely hypothetical and that it may appear somewhat vague, but when we get to the boundary of what is known we must have recourse to provisional hypotheses if investigation is to continue. It is in this spirit that these conclusions in regard to the influence that regulates the growth of new parts are offered.

REGENERATION IN VERTEBRATES.

Let us turn now to the less speculative and purely descriptive side of the problem.

Beginning with one of the lower groups we find that fish have excellent powers of regeneration. The tail regenerates if removed at any level, and in some species even when the end of the vertebral column is also cut off. The lateral fins

that correspond to the limbs of the higher vertebrates will also regenerate if cut off as well as the dorsal and ventral fins. In this connection I may recall an elaborate experiment recently carried out on the Pacific Coast. It has been said that salmon return after their sojourn in the sea to the same rivers in which they were born. In order to test this view, V-shaped pieces were cut out of the tails of thousands of individuals of young fish and parts of the dorsal or ventral fins were also cut off. The fish marked in this way were turned loose in their native streams and their return from the sea awaited. It is needless to point out that the experiment was futile, for whether they returned or not they would regenerate their fins. In order to be sure that these salmon do not behave differently from other fish in regard to their powers of regeneration, I have operated here in the New York Aquarium on two of the species of salmon used and have found that they have the power to regenerate the tail, the dorsal and the ventral fins. As yet I have not determined with certainty whether the adipose fin has the same power or not.

Amongst amphibians, salamanders and newts have long been known to have remarkable powers of regeneration. Spallanzani cut off all four legs and the tail six successive times and each time new parts regenerated. He calculated for a single individual that, in all, 647 new bones must have been formed in the course of a single summer. The last time the legs regenerated as quickly as the first.

The eyes also of the salamander regenerate as long as a piece of the optic bulb remains attached to the nerve. In recent years the experiments of Colucci and of Gustav Wolff have shown that the lens of the regenerating eye does not come from the skin as it does in the embryo, but from the upper edge of the iris. We thus see that an organ may regenerate from a part of the body from which it is never derived in the embryonic development.

One of the most novel of the recent experiments with salamanders is that of Tornier. He has shown how we can produce in the salamander, at will, a supernumerary leg. By cutting

through the skin and the muscles at the side of the leg, and at the same time wounding the bone beneath, a new leg develops on the side of the old one. Unless the periosteum of the bone is injured nothing occurs. This shows that the material derived from the periosteum is the most important element in the formation of the new limb, and other results support this conclusion. In still another way a double limb may be produced. If the leg is first cut off and, after the regeneration has begun, a ligature is tied over the new tissue so that it becomes constricted into two parts lengthwise each will produce a new foot. Tornier suggests that the double limbs sometimes found in human embryos may be caused by folds of some of the membranes constricting the limb bud at an early stage of its growth.

At this point it may not be out of place to say a few words about the histologic changes that take place in the regeneration of a new part. The process has been more carefully studied in the tail of the salamander than in any other animal. When the tail is cut off the skin grows over the cut surface in the course of a few days. Those parts of the muscles that lie near the cut end begin to break up. The muscular tissue, as such, disappears, and the protoplasm forms a ball around each nucleus. These are the so-called sarcoblasts, and out of some of them the new muscle-tissue is formed. A little later, or at the same time, the periosteum of the bone begins to thicken, and soon sends forward a cord of cells from the cut end of the bone into the new part. The segregation of the material of this periosteal cord now takes place and the centers of the new bones are laid down. The muscle fibres begin to develop out of the sarcoblasts; the nerve extends into the new structure, and the blood vessels also send out branches into the part. These changes continuing, the elements of the new limb are formed. All these events must take place synchronously in order that a perfect limb develop. Should any one of the component parts lag too far behind the others a normal product will not be formed. I shall return to this question again.

One of the most striking cases of the artificial production

of supernumerary parts is that which Tornier has recently described for the tadpole of the frog. By a suitable operation a frog with four or even with six hind legs can be made. The method of operating was as follows:—Young tadpoles were selected in which the beginnings of the hind legs were present as small knobs, one on each side of the base of the tail. With a pair of scissors the tail was partially severed at the base so that each leg rudiment was cut into two parts. As subsequent results showed, the rudiment of the pelvis was also cut in two by the same operation. In consequence either two pelves developed and four legs, or in some cases three pelves (or their equivalents) and six legs. The results depend on the great powers of regeneration shown by the severed rudiment of the pelvis and legs.

Barfurth has shown that the hind legs of older tadpoles have the power to regenerate, but after the tadpole has changed into the frog this power is rather suddenly lost. It has been generally assumed that none of the legs of the adult frog has the power to regenerate, but I have found that this is not always the case. In two instances I have seen a frog regenerate a new, imperfect fore-leg, but only after several months, and Mr. Goldfarb, who has been working with me, has obtained the same result. This occasional regeneration, imperfect though it be, shows that the power to replace lost parts is still to some extent present in the adult frog, so that it is one of the best subjects for future experiments in attempting to induce regeneration by artificial means. I have already carried out many experiments with this end in view, so far without much success, but enough has been seen to indicate that the quest is far from hopeless.

Passing now to the higher group of reptiles we find that lizards can regenerate the tail, but not the legs. The new tail is imperfect, however, inasmuch as the vertebral column is replaced by only a cartilaginous tube containing a thin filament extending from the end of the old nerve cord. Double-tailed lizards are not infrequently found and can be produced artificially by making a wound in the side of the old tail.

In birds regeneration of new parts is still further limited, the beak alone of external organs having the power to regenerate if broken off.

Finally in the mammals neither the limbs, tail nor other external organs have the power to regenerate if lost.

Thus as we ascend the vertebrate scale we find the power of regeneration diminishing.

What is the cause for this loss? Some zoologists seem inclined to believe it to be due to increasing complication of structure, but I do not think this can be true. The eye of the salamander is a very complicated organ, and yet it regenerates from a piece only of the bulb. Other zoologists, of whom Weismann is the most noted example, believe that the power to regenerate is something that has been acquired by natural selection in those animals most subject to injury or in those parts of the body most often destroyed. This view I hold to be utterly erroneous. To give but a single case in point:—The eye of the salamander is an organ that is seldom or never injured unless the animal itself is destroyed, yet as we have seen, it has astonishing powers of regeneration. If, then, neither complication of structure nor natural selection will explain why some vertebrates regenerate and others do not, is there any other explanation that can be offered? If, as I think probable, the power of regeneration is closely related to the power of growth, inherent in the protoplasm, why should this power be lacking in certain forms?

WHY CAN NOT MAN REGENERATE AN ARM OR A LEG?

For several years I have been making experiments and examining this question in various ways. I do not feel that I can give yet a satisfactory answer, but the evidence indicates, I think, with some probability, that the failure is due to the fact that the different tissues have very different rates of regeneration. In other words, each tissue in man seems to possess the power to regenerate its kind, but not all at the same pace, hence they fail to co-operate at the proper time to form a new structure. In man the skin regenerates; the muscles

regenerate, though less well, perhaps; the nerves and the blood vessels regenerate, and the bones even have a not inconsiderable power to mend and even to some extent to regenerate. Hence, as I have said, the failure of the new limb to develop does not appear to be due to the failure of the individual elements to regenerate, but is due to their failure to regenerate concurrently. The bones or the nerves or the muscles may be the main cause of the trouble, for they produce new material with great slowness.

In this connection it is instructive to observe that in the vertebrate series the failure to regenerate is found in cases in which cartilage begins to change into bone. Within the group of amphibians we find this change taking place. The newts and salamanders, with partly-cartilaginous bones, regenerate readily, and so do the larval frogs, while in the adult frogs, where the bones have become harder, regeneration has almost disappeared. In the lizard the power to regenerate the leg has been lost, but it can regenerate its tail; and the tail vertebræ are less hardened than the bones of the leg.

I do not wish to affirm that the lack of co-ordinate regeneration is the only cause of the failure to regenerate in higher vertebrates, including man, but, as I have already said, there is some indication that the main trouble lies in the slowness of certain tissues to regenerate in time with the other tissues. But if the tissues in man still possess the power to regenerate may we not hope in time so to adjust their rate of regeneration that the replacement of a lost limb may be induced? I can not but think that some day this may be accomplished.

ON THE NATURE AND CAUSE OF OLD AGE^{*}

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NOTHING is seemingly more familiar to us than the change from youth to old age. The limitations which befall the old are conspicuous. The enfeeblement of years we all know, and there is probably no one in this audience who has not already experienced something of the impairment of powers which the years bring. But these signs of change which we observe in others and experience in ourselves are merely superficial and external, and though we may describe something of the alteration of the shapes of organs, something of the hardening of the tissues, something of the generative change, still we do not get from any study of the very old a clue as to that which is essential as the cause of these phenomena. Nor is it to be expected since the condition in extreme old age is, so to speak, merely the last term of a long series, that we should by the study of that term alone arrive at an understanding of its nature and origin; still less, an understanding of its cause. It is obviously more scientific to study the whole series of terms because we know that ageing is going on in the young as well as in the old, and it needs but a superficial consideration of the facts to convince us that the problem of growing old is one which concerns the entire period of life. The studies which I have made on this subject, which have extended over a number of years, have led me to the conviction that the clue is to be sought rather in early stages of development than in later, and I shall accordingly deal with the subject from the broad biological standpoint; indeed, you will not expect me to

^{*} Lecture delivered February 24, 1906.

add another to the long list of literary descriptions of the losses and the joys of old age. We all honor Cicero, but we should hardly consider him a biologist on account of his essay, "*De Senectute*."

A few words of preliminary explanation are your due. The results and opinions which I have to present to you on this occasion are chiefly personal, and based upon my own observations. They have not yet gone before the court of scientific judgment and been subject to discussion and criticism by others. As you know, it is not until after such discussion that we can expect new views to have acquired a definite form and a positive scientific value. It will therefore be desirable to exercise your critical faculties in considering the matter which I am to lay before you.

The material I have to present falls naturally into two divisions, and we shall take up first, the consideration of certain laws of growth; second, the study of certain changes in cells and tissues during development. I shall then try to show that these two sets of phenomena are intimately correlated, and that their correlation affords us a conception of the essential changes, the final result of which is old age. In other words, I shall offer to you a cytological explanation of senescence.

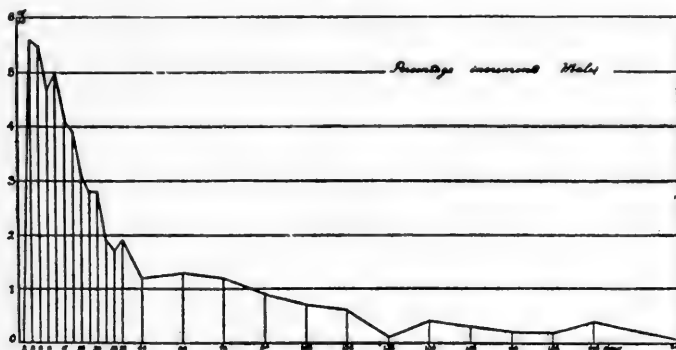
We will give our attention first to certain phenomena of growth. I should prefer to deal with man, but although we have very extensive statistics of the growth of man, they are all, so far as known to me, more or less incomplete, and we still lack adequate statistics of the growth of children from one to six years, which is precisely the period which for my purposes is of especial importance. The statistics which I myself have published in regard to the growth of guinea pigs are still from a biological point of view the most complete which we possess for any species. I chose guinea pigs for my observations from purely practical considerations, as they are easily kept without great expense, and owing to the extreme variabilities of the markings, the individuals can easily be identified. The results of my investigations were published in 1891 in the *English Journal of Physiology*. The first neces-

sity for us is to gain a correct conception of the true *rate* of growth. The majority of writers have used this term in a sense which seems to be not correct, but rather misleading. The usual practice has been to compare the absolute amount of weight added during a given unit of time, with the actual absolute increments of equally successive periods. Now a little consideration shows us that the absolute increments do not really correspond to the rate of growth, for during each period the size of the body increases. If the absolute increments remain the same throughout that would mean a relatively enormous increase in the dimensions of the animal, while it was young, and relatively slight increase when it was old; or if, as occurs actually in the course of development of mammals, the absolute increments during young periods are much smaller than the absolute increments during more advanced periods,—such a contrast exists between a child at birth and a child at fourteen,—then by such a measure we should say that the rate of growth had increased. In reality this view is indefensible, for if the body increases in size and the rate of growth is constant, the proportionate increment would remain the same, but the absolute increment would become steadily larger. Reciprocally, it is evident from this, that if the absolute increments are constant the rate of growth diminishes.

After deliberating on the problem for some time, it seemed to me that the most convenient way of representing the rate of growth was to calculate the percentage of increment occurring in one day based on the weight at the beginning of a period. It is evident that the increase of weight depends upon two factors: First, upon the amount of body substance, or in other words, of growing material present at a given time; second, upon the rapidity with which that amount increases itself. Hence, for a given period the rate of growth may be expressed as the fraction of weight added during that period. This I have done, expressing the fractional increase in percentages. The tables giving the exact values for guinea pigs, male and female, for nearly three years of growth, have been

printed in the article referred to. On this occasion I think it will be more instructive for you if we take advantage of the lantern and project upon the screen a graphic representation of the results. The curve shown to you in Figure 1 indicates

FIG. 1.

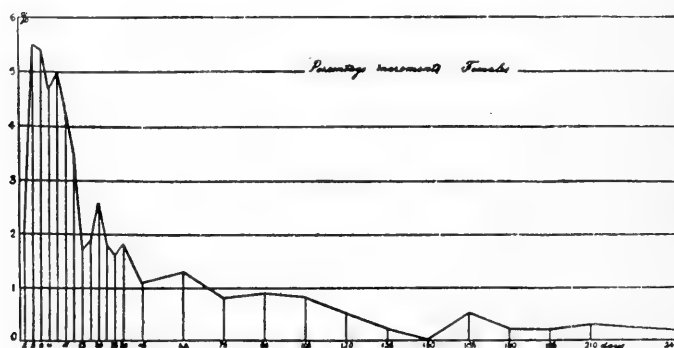


the daily percentage increments for male guinea pigs. Guinea pigs are born, as you know, in a very advanced stage of development, and their birth causes a very marked physiological disturbance, so that they require two or three days to adapt themselves to the conditions of the extra-uterine existence. As soon as they have recovered, we see by the curve that they are able to add from 5 to 6 per cent. to their weight during a single day. Thereafter the percentage which they are able to add each day diminishes, so that by the end of the first month they are able to add only about 2 per cent. in one day. The rate, however, then continues to fall, so that by ninety days it has become less than 1 per cent., and thereafter, as you see readily, still further but slowly diminishes. This curve is highly characteristic and very instructive. It shows, to be sure, a number of minor fluctuations which are probably purely accidental, and quite without biological significance, but due solely to the limited extent of the statistical material. The fluctuations are so small that they do not in any way obscure the general course of the curve. Its main characteristic is that the decline in the percentage increments is very rapid at first; then gradually becomes slower and slower, at last becoming very gradual indeed. We may regard

the percentage increments as the indices of the power of growth, and hence we may express the general fact to which I have called your attention thus: The power of growth diminishes very rapidly at first, gradually more slowly, and the diminution as the animal approaches maturity becomes very slow and prolonged.

The next curve, Figure 2, is the corresponding one for the females. The values are here very similar, and the general course of the curve is the same as for the males. You will notice that for the females also, as soon as they have recovered from the shock of birth, the rate is between 5 and 6 per

FIG. 2.

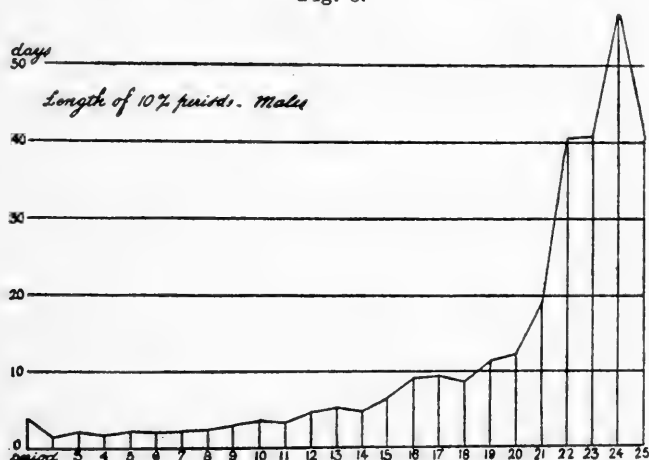


cent.; that it falls during the first thirty days to about 2 per cent.; by ninety days has fallen below 1 per cent., and thereafter slowly diminishes up to the two hundred and forty-first day, which is as far as the curve proceeds. The conclusion which we deduced from an examination of the curve of the male is therefore entirely confirmed by the study of the curve of the female, and the conclusion which we drew, that the decline in the rate of power of growth is most rapid in the young animal and less rapid in the old animal, is clearly confirmed.

These facts can be conveniently represented in a curve of another form. On the basis of the accumulated statistics I have calculated the length of time required by guinea pigs to make successive additions of 10 per cent. to their weight, and by plotting these results for the two sexes I have obtained

the curves to which I will next call your attention (Figures 3 and 4). These represent graphically twenty-five successive additions of 10 per cent. each to the weight of the guinea pig. The ordinates represent the number of days required. As you will observe at once, the early additions of 10 per cent. each are made very rapidly, and it is not until we get to the seventeenth addition that we find nine days or more necessary to make it; and by the time we get to the twenty-second addition, it takes some forty days. The curves, as you will notice, for the later additions are somewhat irregular. This is due to insufficient statistical basis. The insufficient statistics came

Fig. 3.

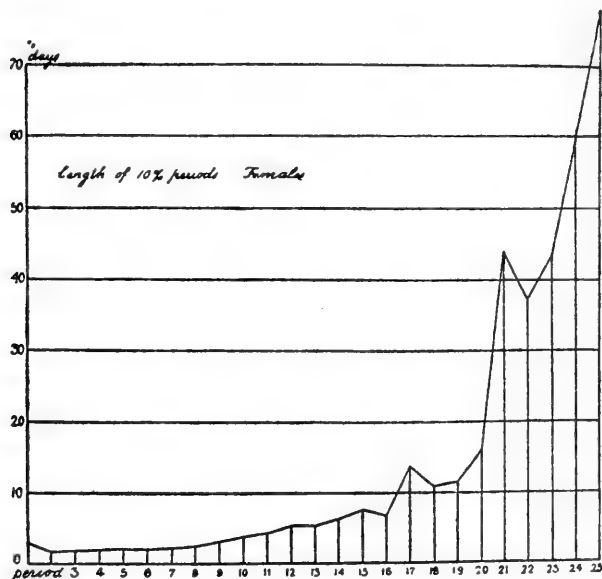


about because I had only a limited number of guinea pigs, and some of them died off from time to time so that for the older stages I had a comparatively small number of observations.

It may interest you, too, to hear of the accident by which my accumulation of statistics was abruptly terminated. For greater safety my animals were kept in a locked room, the floor of which was divided into large and commodious pens. I deemed myself safe from dogs, but unfortunately the janitor of the building had a bull terrier bitch which he wished to isolate for two or three days from other dogs, and therefore chained her up in the animal room. During the first night

the terrier broke loose and the next morning we found ninety-four guinea pigs dead, leaving only four alive of all those which I had kept and raised for a long period, making as I went along, a careful biological record of each individual. The work of nearly five years was thus suddenly ended. Fortunately the data already in hand proved sufficient, as I think the curves

FIG. 4.



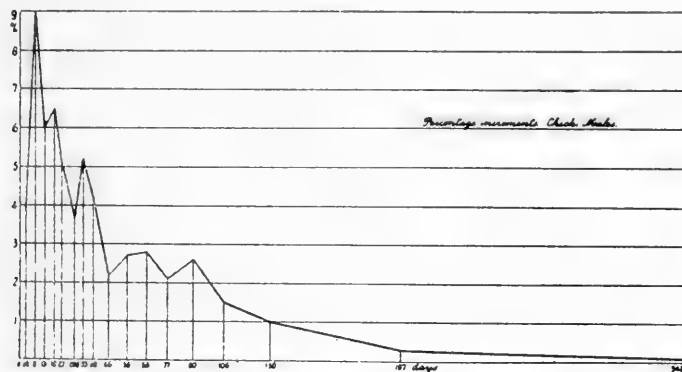
demonstrate, to bring out clearly the general character of the increase in these 10 per cent. periods.

In addition to the observations on guinea pigs, I have some upon the growth of chicks which have not yet been published. The observations were made upon a very small number of individuals, but nevertheless they suffice to confirm the general results obtained from the study of the growth of guinea pigs.

Chicks, like guinea pigs, are born in an advanced stage of development, and there occurs in them an absolute loss of weight during the first day after hatching, but by the fourth or fifth day they appear to entirely recover. The daily percentage increase is greater than in the guinea pig. In the period from the sixth to the tenth day inclusive, the average is nearly or quite 9 per cent. (Figs. 5 and 6). Thereafter it falls

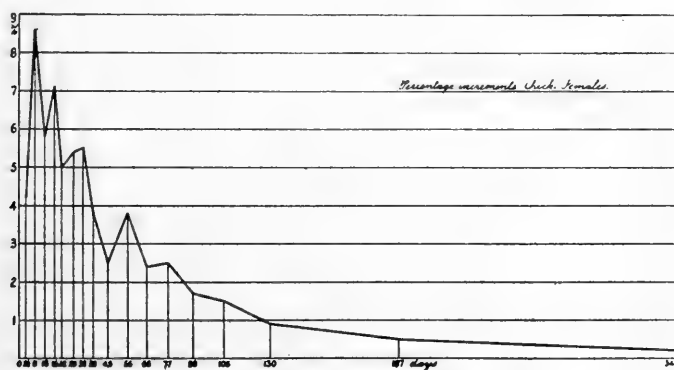
off very rapidly, and at the end of the third month reaches about 2 per cent., and later declines gradually and slowly. A comparison of the curves presented in Figures 5 and 6, with the corresponding curves of the guinea pigs, makes the general similarity obvious.

FIG. 5.



It is evident that in these two species the younger the individual the higher its percentage increment of daily growth. If we go back in the history of these animals, we should expect to find a still more rapid growth. Now this we can do to some extent by utilizing the statistics of the growth of rabbits, animals which are nearly related to guinea pigs, but differ from them

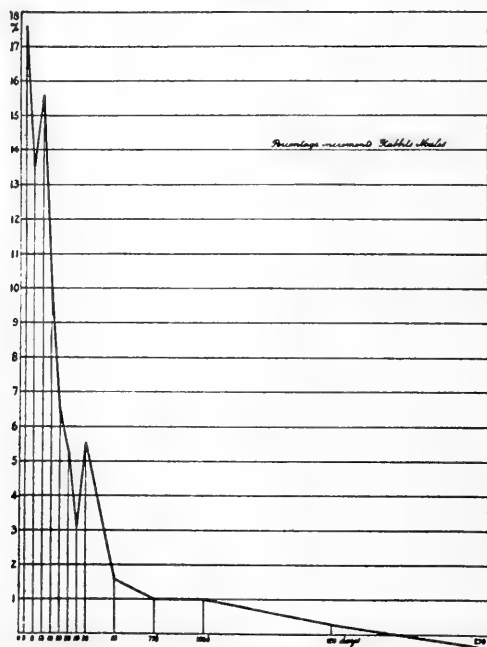
FIG. 6.



in being born in a very immature condition. For the rabbits also I have only a very limited number of observations, but they suffice to show that our expectation is realized, and that the rabbits being less developed at birth than the guinea pigs

grow much more rapidly. The average for the males for the first five days of growth is, as you see (Figs. 7 and 8), 17.6 per cent.; for females 16 per cent.; for both sexes, the decline in the percentage increment after birth occurs with enormous rapidity, taking place much faster than does the decline in guinea pigs. Rabbits in about thirty days reach a stage of development nearly similar to that which the guinea pig shows at birth, and it is interesting to note that when the

FIG. 7.

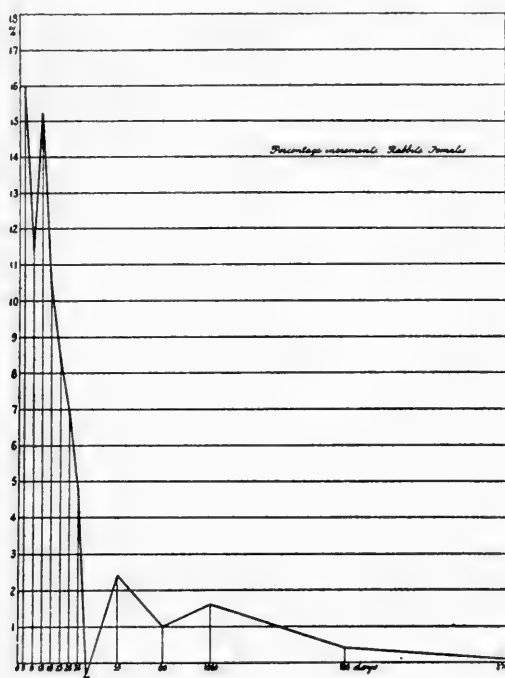


rabbit is thirty days old it has about the same daily percentage increment as the new-born guinea pig.

The facts to which I have called your attention, direct our interest next towards the study of the growth during the foetal period. I regret to say that I have as yet no satisfactory data in regard to this, though I had hoped to be able to accumulate them before this time. We can, however, get some general impressions by the inspection of the normal plates of development which have been issued under the editorship of Professor Keibel. In two of these normal plates, namely, those of the chick and of the rabbit, the age of the embryos is recorded. I

therefore have had lantern slides prepared of these plates, and as you examine the pictures you will readily see the very rapid diminution in the size of the embryo as we proceed from the older to the younger stages, and if you estimate, without making any absolute measurements, it will at once be evident that a chick of one day or a rabbit of eight days must multiply its bulk many hundredfold during a comparatively short period, and it needs only this general comparison of sizes to satisfy one's self that the rate of growth of both these species

FIG. 8.



during the foetal period must be enormously more rapid than it is after birth, for it is evident that the chick, after hatching, does not increase its weight many hundredfold, nor does the rabbit at birth proceed to increase its weight many hundredfold to attain the adult size. There is, therefore, unquestionably a great contrast between the very high speed of development during the early stages of the embryo and the speed after birth.

In order to get at least some approximate values for the rate of foetal growth, I have attempted to utilize the preserved

specimens of rabbit embryos of known ages in my laboratory. These were all preserved in Zenker's fluid, and then kept in 80 per cent. alcohol. I had gathered, for the purpose of getting material for the normal plates of the rabbit above mentioned, a very considerable number of such embryos, and had selected for the different ages in days embryos, which, by comparison of series of different ages with one another, seemed to be typical in size and form for each given day. I thus had at my disposal a series of embryos more or less truly normal and typical for each successive day of development. These embryos I then proceeded to weigh. Such weights, of course, are not accurate, and could not be relied upon to indicate the true weight of the living embryo, yet we probably can use them for purposes of comparison with one another without danger of erroneous conclusion. At ten days the weight is only 5 mg., but such specimens cannot be accurately weighed because the alcohol evaporates rapidly, so that this value is at best approximate only. But at fifteen days I found a weight of 176 mg. This indicates an increase in five days of 3,520 per cent., or an average of 704 per cent. daily. At twenty days I found a weight of 1,860 mg., making an increase from the fifteenth to the twentieth day of 1,058 per cent., or an average of 212 per cent. daily. We have then this contrast: From ten to fifteen days, a rate of 704 per cent.; from fifteen to twenty days, 212 per cent. The probable error of these determinations is very large; just how large, of course, I cannot at present say. But we are dealing here with values of an entirely different order from those which we have encountered as values for the percentage increments after birth; those values, you will recall, were between 5 and 6 per cent. for the guinea pig and 16 to 17 per cent. for the rabbit. It seems to me, therefore, that we are perfectly safe in saying that the rate of growth during the foetal period is much more rapid than in the post-foetal, and not only that, but it is far more rapid in the early stages of the embryo than in the later, and indeed we may go even further, for taking these figures which I have presented to you as a guide, they indicate that

the further back we go in the history of the individual the more rapid do we find the rate of growth, and since we have encountered in the rabbit in the age from ten to fifteen days an average daily increment of 704 per cent., we probably shall not go far astray if we assume that at a yet earlier stage the rate of increase was at least as high as 1,000 per cent. This seems an enormous value, almost incredible, at first thought, if we base our judgment upon the impressions of the rate of growth which we get from our familiar acquaintance with the growth of children and young animals. If, on the other hand, we turn to the multiplication of bacteria, we learn that such a rate of growth is by no means extraordinary. For, if a bacterium divides once every half hour, and this, as you know, is what may and does often actually occur, then in half an hour that bacterium has grown 100 per cent. At the end of an hour it has grown 400 per cent., and at the end of an hour and a half 800 per cent. This you recognize at once is a far greater speed of growth than that of 1,000 per cent. in one day, which we have assumed as possible for the early stage of the rabbit. We do not venture too far if we regard that estimate as conservative. Now the maximum rate which the rabbit shows after birth occurs immediately after recovery from the post-natal retardation. According to my statistics that rate is 16 per cent. in the females and 17.6 per cent. in the males as the average for the first five days. If we subtract, say 16 per cent. from 1,000 per cent., we get 984 per cent. In other words, this value means that in the case of the rabbit 984 per cent. of the original growth power with which the individual starts is lost at the time of birth. Approximately the same is true of other mammals including man. In all of them only a small fraction of the original growth power still exists at the time of birth. Most of it has been already lost.

This conclusion has a special significance for us, for if we are to regard the loss of growth power, as we have always hitherto done, as one of the most characteristic marks of old age, then it becomes probable that we should find more favorable opportunities for determining the cause of the loss of

growth power by studying the fœtus than by studying the old individual, since it is during the fœtal period that nearly the whole of the loss of the growth power is effected. We thus arrive at the somewhat paradoxical conclusion that in order to investigate the fundamental phenomenon of old age we must specially investigate the very earliest stages of the individual. Now we are accustomed to regard all the performances of the body as due to cells and to their activity, and we consider that the activities of cells are explained by their structure, and we can see, as we all know, with the microscope, some of the points of structure by which cells doing different kinds of work differ from one another. Hence the inquiry immediately presents itself to our minds, can we not see in the cells some changes which we can correlate with the changes in the growth power? This brings us to the second part of my lecture.

I now ask your attention to the phenomenon of cytomorphosis. This is a term which I first proposed in the Middleton-Goldsmith lecture in 1901. It seemed to me very desirable to have a single comprehensive term to designate all the structural modifications which cells or successive generations of cells may undergo from the earliest undifferentiated stage to their final destruction. As I then pointed out, it is convenient, though somewhat arbitrary, to distinguish several successive stages of cytomorphosis; for we have first the undifferentiated stage, second the stage of progressive differentiation which itself often comprises many successive phases, and third the regressive stage, or that during which degeneration or necrobiosis occurs. There seem to me to be two principal types of differentiation which can be distinguished by the terms "*cyto-static*" and "*cyto-dynamic*." In the *cyto-static* differentiation a portion of the protoplasm of the cell is changed permanently as to its chemical constitution and as to its structural arrangement, in order to serve some special purpose. This is illustrated, for instance, in the production of horny material or of fibrillar connective tissue; or again, of bone. The object of such differentiation is to create a relatively permanent disposition of the cell derivatives. On

the other hand, when a cyto-dynamic differentiation occurs the protoplasm may be modified, but still remains protoplasm, and performs certain functions for which it has become specially adapted. Such a differentiation is illustrated for us by the nerve cells, muscle cells, and gland cells. But in both of these types it is to be noticed that the change in the protoplasm is very important, and that the modification of that is an essential part of the cytomorphosis. So much is this the case that attention has rather wandered away from the modifications which the nuclei undergo, and it is only recently that we are beginning to appreciate that nuclei also in the course of the differentiation of tissues undergo cytomorphic changes which are undoubtedly as essential and significant as those which go on in the protoplasm. Now if we examine undifferentiated tissues and compare them with those which have been differentiated, we find that we can readily make a generalization as to one important modification which is common to them all. In the undifferentiated tissue the protoplasmic body is always relatively small; often it appears barely sufficient to clothe the nucleus respectably. In all differentiated tissues, on the other hand, we find that there has been a great increase of protoplasm. In the case of all cells which show the cyto-dynamic change, the increase of the protoplasm in the older stages is very obvious. Dr. Eycleshymer, in one of the most valuable and significant contributions to cytology which has yet been published in America, has given us some statistics as to the proportion of protoplasm and nucleus in the developing muscle fibers of *Necturus*. You are of course all aware that the striated muscle cell originally has but a single nucleus. As it grows the number of nuclei multiply. Eycleshymer has shown that in spite of this nuclear multiplication there is a relative increase in the amount of protoplasm. The unit of volume in these determinations is .000,000,01 of a cubic millimeter. In a *Necturus* embryo of 8 mm. there are per each nucleus 2,737 units; in a 17 mm. embryo, 4,318; in a 26 mm. embryo, 8,272; and in the adult 23 centimeters long, the number of units is 22,679. Each of these values is the average of

five determinations. The above computations show interesting changes in the relative volume of cytoplasm and nuclear material during growth. In the 8 mm. the unit of nuclear material is correlated with two or three units of cytoplasm; in the 17 and 26 mm. embryo, with 5 to 7 units; in the adult, with 20 to 30 units. In other words, as the embryo approaches the adult condition, there is a progressive increase in the amount of cytoplasmic material with the end result that there is twenty to thirty times as much cytoplasm in physiological equilibrium with a given quantity of nuclear material as in the earlier stages.

It might be suspected that this problem would be complicated by the question of the relation of the size of cells and the size of animals. We know, however, that the difference in size of animals of the same species, or which are nearly related to one another, depends rather upon the number of cells than upon their size. One dog is larger than another not because he has bigger cells, but more of them. Exceptions to this rule are offered, to some extent, by the striated muscle fibers, and a very striking exception is offered by the nerve cells, for these apparently, as shown by the excellent investigations of Hardesty, do vary according to the size of animals. The fact to which I have just called your attention of the constant size of cells, makes it of course easier and more certain that we are right in our conclusions that the increase of the protoplasm is a constant and more or less fixed characteristic of advancing development.

I have just now presented to you an estimate of the rate of growth of the rabbit embryo, and we have seen that there is a great difference between the rate from the tenth to the fifteenth day on the one hand, and the fifteenth to the twentieth on the other. I happen to have at my disposal some very good series of the rabbit of ten days and of sixteen and a half days, and with these as a basis of comparison we find that in all the tissues there has been a marked increase in the protoplasm during this period in proportion to the nucleus in each of the cells. It is a very striking and obvious change. I find also

that there is a rich field for study as regards the structure of the nucleus to which I would like to refer in the hopes that someone may be led to pursue further this important line of investigation. In the ten-days rabbit the nuclei of the blood cells and of the endothelium of the blood vessels alone show some differentiation. All the other nuclei in all the parts of the body are more or less similar, round or oval in form, with a rather coarse and somewhat granular network in their interior and two or three rounded, somewhat irregular chromatin granules. At sixteen and a half days the nuclei differ extremely in the different tissues. In the cartilage cells the nuclei have a well defined superficial layer, and a well marked central corpuscle. In connective tissue the nuclei are smaller and have usually two rather small chromatin granules; but in the dermis the chromatin material is scattered more; in the muscles of the heart the nuclei show the chromatin widely distributed and rather a fine network, making a great contrast with the nuclei of the ganglion cells which have at this stage as yet no nucleoli but a coarse network of fine threads with some finer granules at the nodes. So, too, I have noted characteristic differences in the entodermal cells of the œsophagus and trachea, and of those of the liver and the Wolffian tubules. In other words, the nuclear differentiation is already far advanced. It is evident that during this brief period of five and a half days the larger part of the total differentiation of tissues has already been accomplished, and though it is difficult to estimate such a complicated series of changes quantitatively, still I think we shall not go amiss if we say that as much differentiation is accomplished during the short period as during all the remainder of the animal's existence. The differentiation proceeds with enormous rapidity at first and gradually goes on more and more slowly. Here again there is need for a much more exact and thorough investigation than has yet been undertaken, but I believe we are safe in making the generalization that the rate of differentiation is greatest during early periods and that it declines during early foetal periods and declines with great rapidity.

It seems to me that we meet here a principle which is fundamental and which regulates all the changes connected with development in all the higher forms; the principle that change is most rapid in the younger stages, that the rate of change declines more rapidly in the young than in the old, and that it gradually diminishes with ever-increasing slowness. I think this can be exemplified also, it is interesting to note, in the mental history of the individual. The child at birth has a brain not yet fully differentiated, and which must be, as regards impressions and ideas, very nearly a perfect blank. During the first year the child learns all the great adaptations of life, the great facts of existence, the principal phenomena of the physical world, and the foundations of all human ties. It acquires the great metaphysical conceptions of time, space, and the ego. In brief, it is probably no exaggeration to say that a child learns more during the first year of its existence than it learns in all the subsequent years of its life, and that from the time of birth the power of learning is rapidly declining. It declines very fast during infancy, more slowly during childhood, very slowly in the adult, but before mid-childhood is reached the most of the power of learning is gone. I find myself led by such considerations as these to differ somewhat from Dr. Osler, as his opinion implies rather a critical change occurring at a certain definite time. As a biologist, I think the alterations which occur in the life of an individual are progressive and gradual and my observations of the individuals of my acquaintance have led me to think that it is only by exception rather than by rule that the change from a productive to an unproductive mental condition takes place abruptly. In placing his age limit at 40 I think Dr. Osler has indulged in an amiable exaggeration. It may be true that that age marks in intellectual men usually a transition or the point where the accumulated losses which have been occurring from birth on reveal their effects clearly, but in the great majority of men comparative mental fixity surely occurs at a much earlier period. If you will allow me to wander for a moment from the strict discussion of our immediate theme, I should like to refer to

what may be called the theory of permanent mental fatigue. The organic changes which go on in the nervous system diminish its pliability and there comes a time when the individual finds it exceedingly difficult to bring his mind into any unaccustomed form of activity. How completely we are mastered by this difficulty is often hidden, I believe, from our recognition and from that of our friends, because we have acquired certain habits of activity which we are able to keep up, but we are not able without ever-increasing difficulty to turn to new forms of mental activity, or in other words, to learn new things. When we grow old we may still continue to do well the kind of thing which we have learned to do, whether it be paying out bills at a bank or paying out a particular set of scientific ideas to a class of students. If we try to overstep the limits of our acquired expertness we find that we are held up by this sense of permanent mental fatigue. Usually this condition comes about gradually, but I have known, as I presume you all have, several cases in which it has appeared suddenly, where a man who up to a certain time was fond of mental exertion suddenly ceased to be mentally active. We have probable illustrations of this in the careers of well-known scientific men, which was perhaps the case with Theodor Schwann, who, after having founded the cell theory for animals, lived for more than forty years unproductive and almost unknown. I think the theory of permanent mental fatigue, in connection with the theory of gradual decline which we are considering this evening, could be usefully developed and might well be utilized by the psychologists in their studies. But let us return to our proper subject.

In the first part of our lecture we have considered the rate of growth. In the second part we have considered the rate of differentiation. You are probably all prepared in your minds for the conclusion which I wish to put forth, that the two series of phenomena are intimately correlated with one another; that they are indeed merely two aspects of one thing. I may express the correlation in other terms, perhaps more apt, by saying that I regard the loss in the power of growth of the

body as due to the increase and differentiation of the protoplasm in the single cell. Of course this increase in the cells does not go on alike in all. In some it is precocious; in others it is long retarded. An important part of the functions of our body depends upon the lifelong preservation of certain cells in more or less embryonic condition, and capable therefore of constant multiplication. Such cells cause the growth of the hair and nails. Such cells serve for the renewal of the blood, and so on. But broadly speaking, it is of course true that the amount of protoplasm increases in proportion to the nuclei and that as it increases the growth power diminishes. Are we not therefore justified in saying that the increase and the differentiation of the protoplasm is the cause of the decline of the power of growth? Growing old, in other words, consists primarily in an increase in the proportion of protoplasm. We thus have a cytological mark by which old age can be distinguished, and we are able to connect senescence with visible changes in cells:—we are able to say there is a histological basis or cause of old age.

Professor Metschnikoff has published a work called "*The Nature of Man*," which I presume is known to most of you. It is a very agreeable book to read and reveals certainly an attractive and interesting personality in its author. He wanders charmingly past Greek and medieval philosophies, past great events of modern science, and concludes that all of these are more or less unsatisfactory, nor does he get more content out of the various religions of the world, but finds in the maladjustments of the body and in the necessity of growing old grave sources of misfortune. He is able, however, to reach an optimistic conclusion because he finds that among the maladjustments is that of excessive size of the large intestine in consequence of which fermentation is peculiarly apt to occur in that organ, resulting in the production of poisonous materials which, being absorbed into the system, become the cause of the depressing symptoms by which the old are especially afflicted, and in connection with this there occurs a great activity of the phagocytes, and he finds in the malign activity

of these an essential characteristic of age. As a remedy for all these ills he takes advantage of the discovery claimed by Professor Bienstock that lactic acid interferes with the fermentation processes in the large intestine. He therefore proposes, apparently as the substitute for philosophy and religion, the regular drinking of sour milk. Surely such a method of dealing with the phenomenon of old age should not have our sympathy. We cannot attribute to peculiarities in the rectum or to the activity of phagocytes, those phenomena of old age which, if the views I have been presenting to you this evening are correct, must be regarded as the accumulated results of incessant changes, most of which have gone on from the very youngest stages of our existence, while only a very small minority of them go on during what we call our old age. It seems to me thoroughly unscientific to attribute to incidental phenomena the importance which ought by right to be attributed only to a long series of cytomorphic changes which go on from the moment of the procreation of the new individual up to its final failure. Nor should we be frightened from a sound scientific conclusion because it takes a somewhat paradoxical form, for if I have argued correctly, the younger and earlier the stage the more rapid is the decline, and a characteristic of old age is that it is the period when decline is slowest.

There is another aspect of the problem to which I should like to call your attention briefly. If senescence consists in the relative increase of protoplasm, then we shall expect to find that the opposite also would be true, and that growing young or rejuvenation would consist in the over-production of nuclear material. Now, of course, it is through the fertilization of the ovum that the typical young individual is produced. The ovum is a relatively large structure, much larger than any other cell which we know, and it contains but a single nucleus, or after its maturation, so to speak, half a nucleus. When it is fertilized development begins and soon the young organism with embryonic cells is produced. If now we follow with our present question in mind the changes which go on, we see that the ovum without increasing essentially its total volume,

changes from a single cell into a number of cells. This we call the process of segmentation. Each of these cells, however, is provided with a nucleus, and each nucleus is of the full size, and with a full stock of chromatin. In other words, it is evident that during the process of segmentation there is a very rapid increase in the amount of nuclear material without a corresponding increase in the protoplasmic material. The immediate result of the beginning of development is then the multiplication of nuclei which goes on until there is only a small proportion of protoplasm left for each nucleus. It seems to me, therefore, that we are justified in looking upon the process of the segmentation of the ovum as a process of rejuvenation, and that we are further justified in saying that as senescence consists in the excessive growth of protoplasm, so conversely, rejuvenation consists in the excessive growth of nuclei, and with that we have reached, in my opinion, a well-rounded and completed theory of growth and cytomorphosis.

In conclusion, let me point out that in the lowest organisms we have probably no such phenomenon as growing old, no alternations of senescence and rejuvenation; but in order to evolve the higher organisms, in order to evolve man himself, it has been necessary that differentiation should be introduced, and differentiation involves the gradual loss of the growth power, and the impairment of the cells. It involves, as a further result, the ultimate death of the individual. The evolution of a higher type brings with it differentiation and death in combination. To the differentiation which has been accomplished through long, unmeasured periods of time, we owe the faculties and privileges which we enjoy, our power of thought, our vast stock of emotions and memories, our privilege of scientific investigation, and all those great and varied resources which make possible to each of us a life rich, manifold and interesting, but the price we must all pay for these privileges is death.

MODERN VIEWS REGARDING PLACENTATION*

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DURING the last thirty years the human placenta has formed the subject of many researches, but it is only within very recent years that its real structure and phylogenetic relationships have been established. Indeed, there is no sphere of embryological inquiry in which more dissension has arisen, not only in regard to the collection of data, but also in the interpretation of them. Some of the errors of pioneer workers are undoubtedly attributed to crudity in microscopic technic, but the mistakes of recent investigators equipped with modern facilities must be otherwise explained. Hasty generalizations based on the study of scanty material are largely to blame for many of the misinterpretations of histologic appearances which have been made. Our knowledge of the structure and development of the human placenta would never have been satisfactory, so long as the earliest stages of gestation had remained unknown.

When Peters, of Vienna, in 1899 published his splendid monograph on the human uterus and ovum during the first week of pregnancy the all-important link was completed in the chain of evidence which was being slowly forged, and a standard was established by which all previous investigations could be judged. Moreover, it was then for the first time made possible to establish an accurate comparative study of the human placenta, by which its true phylogenetic relationships might be determined. Due credit must always be given to those investigators who, before the appearance of Peters'

* Lecture delivered March 3, 1906.

work, had described early specimens of human pregnancy so accurately as to give them an historical importance scarcely secondary to that of Peters.

In the field of comparative placental study, the investigations of Hubrecht have established a new conception of the early relationships of the mammalian ovum and uterus, which subsequent work has only tended to corroborate. His work on the placentation of the hedgehog must ever be regarded as a classic, not only because of its descriptive excellence, but because it provides the key for the study of placentation in the highest mammalian forms.

Comparative.—In studying those orders of the mammalia in which a relationship is established between the ovum and the uterus, it may be stated that a common feature in the earliest stages is the application of the epiblastic covering of the blastocyst to the uterine mucosa. Various degrees of complexity characterize this relationship.

In the more advanced stages, the chief differences are established, and are to be found in the nature and duration of the relationship between maternal tissues and ovum, the fetal origin of the placental vessels, the reactionary changes in the uterine mucosa, the extent of tissues shed at birth, etc.

Thus, among the marsupials during the short intrauterine existence of the ovum, the non-embryonic portion of the blastocyst develops villi through which nourishment is absorbed from the uterine mucosa. The villi are vascularized by branches of the vitelline vessels after the blending of the yolk-sac with the blastocyst wall, neither the allantois nor the allantoic vessels taking any part in the process. There is no well-marked placental development such as is characteristic of gestation in all the orders higher than the Marsupalia.

In three of these orders, viz., Rodentia, Insectivora, and Cheiroptera, there are two stages of placentation, one, the primary, being that which is formed by a portion of the blastocyst vascularized by the vessels of the yolk-sac, the other, secondary and permanent, that formed by another portion of the blastocyst vascularized by the allantoic vessels. The genera

and species of these orders have not yet been sufficiently investigated to enable us to state to what extent variations from this arrangement exist. It is probable that in many species there is a very imperfect vitelline placenta. In *Sorex*, one of the *Insectivora* for example, Hubrecht has shown that while there is a proliferation of the epiblastic covering of the blastocyst in the region of the yolk-sac, true villi are not formed.

In the seven other orders, viz., *Edentata*, *Ungulata*, *Carnivora*, *Sirenia*, *Cetacea*, *Lemuroidea*, *Anthropoidea*, the placenta is formed at the portion of the blastocyst which is vascularized by the allantoic vessels. In the great majority of cases there is no primary development of villi vascularized by the vitelline vessels, the yolk-sac remaining small or disappearing. Indeed, in many mammals there is no mesodermic covering of the yolk-sac, nor any vascularization of its wall. That a permanent vitelline placenta may, however, be sometimes developed, even in the human female, cannot be doubted.

Colomiatti first suggested this in 1880 as the result of an investigation of certain human monstrosities. Later, Weigert, Labougle and Regnier, and others formed a similar opinion. Corroborative evidence has been more recently afforded by Ballantyne. He has made a careful study of sympodial monsters in which the bladder and lower end of the intestine are wanting and finds that in such cases the umbilical cord is always anomalous, containing only one vein and one artery, the latter arising directly from the aorta at about the level of the second lumbar vertebra. The hypogastric arteries, being absent or rudimentary, do not contribute to the vascularization of the placenta, nor can any allantoic tissue enter into the formation of this organ. On the other hand, vitelline derivatives are present, and from the relationship to the umbilical artery, it cannot be doubted that the latter is derived from the omphalo-mesenteric. Hence, in such cases, the placenta has been termed "vitelline" by Ballantyne.

In various other forms of monstrosities also a similar development may take place. These facts are of the highest importance in helping to discredit the long held view that the allantois is

necessary to the formation of the human placenta. Indeed, apart from this evidence, embryological investigation is quite conclusive on this point. His first showed that the stalk of tissue by which the early embryo is attached to the wall of the blastocyst, and which had previously been known as the allantoic stalk, was not an outgrowth of the allantois to the fetus, but was merely the last bond of union existing after the differentiation of the early embryo from the rest of the blastocyst. At first this stalk is situated at the posterior end of the embryo, gradually being displaced to the ventral surface as the embryo enlarges. In this ventral stalk (*bauchstiel*) the small allantois may be found as a small tube derived from the posterior gut, extending along the anterior pelvic wall. The hypogastric arteries, derived from the internal iliacs, grow outward in the mesoblastic covering of the allantois, through the ventral stalk to reach the mesoblastic layer of the wall of the blastocyst (*chorion*) in which they ramify to form the vessels of the villi. The tube of the allantois never extends to such a distance and does not come into relationship with the chorionic membrane. The mesoblastic layer of the latter is, therefore, not derived from the allantois. It exists before the umbilical vessels reach it.

Neither in cases of vitelline placenta, in which the omphalo-mesenteric vessels supply the villi, is the mesoblastic tissue of the yolk-sac necessary to the chorion. The latter, consisting of epiblast and mesoblast, has all the elements necessary to the formation of the placenta except the blood-vessels, and these are derived from the embryo.

It may, then, be confidently stated, from the investigations which have thus far been made, that the placenta whether temporary or permanent, whether vascularized by the omphalo-mesenteric or hypogastric vessels, is an organ of the non-embryonic portion of the blastocyst. The latter structure has been described under different names, leading to much misunderstanding. The following table gives the terminology of various authors:—

TABULAR COMPARISON OF THE NOMENCLATURE OF CERTAIN FETAL AND MATERNAL STRUCTURES THAT TAKE
PART IN THE PLACENTATION OF THE MAMMALIA.

Hubrecht.	Frommel.	van Beneden.	Fleischmann.	Selenka.	Bonnet.	Balfour.	von Baer.
Trophoblast	Exochorion primitivum + decidualsehicht	Cytoblast and plasmoblast (horseshoe-shaped proliferation)	Chorion . .	Exochorion Träger p. p. Deckzellenschicht (Rauher's and Reichert's cells)	Amniogenese chorion Primitive chorion . .	Epiblast of non-embryonic part of blastodermic vesicle	Exochorion
Diplotrophoblast . .	Membrana chorii + Exochorion primitivum p. p.	Sereuse de von Baer	Seröse Hülle resp. Chorion	Subzonal membrane	Seröse Hülle	
Allantoidean Diplotrophoblast	Allanto-chorion	Allantois chorion, Euehorion, Placentar chorion	Allantoischorion, Gefässchorion . .	True chorion	Chorion
Omphaloidean Diplotrophoblast	Omphalo-chorion	Dottersackchorion, Pseudochorion
Trophospongia . . .	Gefässschicht. Epithel-lager innerhalb der (decidualen) Faserschicht
Wall of blastocyst (independently of histological constitution)	Chorion

To the above list may be added Kölliker's "Ektodermwulst" and Duval's "formation ectoplacentaire" as describing the trophoblast of Hubrecht.

The writer thinks it advisable to follow Hubrecht's advice, restricting the term "chorion" to the description of the human ovum and to employ the following terminology in mammalian embryology generally:—

Trophoblast, the outer epiblastic layer of the wall of the blastocyst, which is applied to the uterine mucosa.

Diplotrophoblast, the former layer, combined with the early mesoblast layer on its inner surface. That part which is vascularized by the omphalomesenteric vessels is termed *omphaloidean diplotrophoblast*; that vascularized by the hypogastric vessels, the *allantoidean diplotrophoblast*.

Within recent years our views regarding the phylogenetic relationships of the human placenta have undergone considerable modifications. For a considerable period the teaching of Sir William Turner has been widely accepted, viz., that the complex chorionic development of the human placenta is found in its simplest stage in the chorionic wart of the pig; next above such forms as the latter being the arrangement found in the ruminants, still higher that in carnivora, and highest of all, that found in the anthropoidea. Hubrecht has strongly urged the intrinsic improbability of the view that the various stages of the evolution of the human placenta should be traced through orders so divergent and specialized as those just mentioned, which are not related in a direct line. Huxley pointed out in 1880 that the various existing mammalian orders tend toward lower ancestors from which the Insectivora have diverged less than other orders, and he considered this order as among the most primitive monodelphian mammals. This view has been supported by many zoological and palæontological investigations. Hubrecht's extensive studies of placentation have further supported Huxley's views, particularly in regard to the central position occupied by the common hedgehog (*Erinaceus Europæus*) among the Insectivora. His studies of the development of the placenta in this animal led to speculations regarding the human placenta which have been fully substantiated by recent workers.

In the hedgehog Hubrecht found that at an early stage the

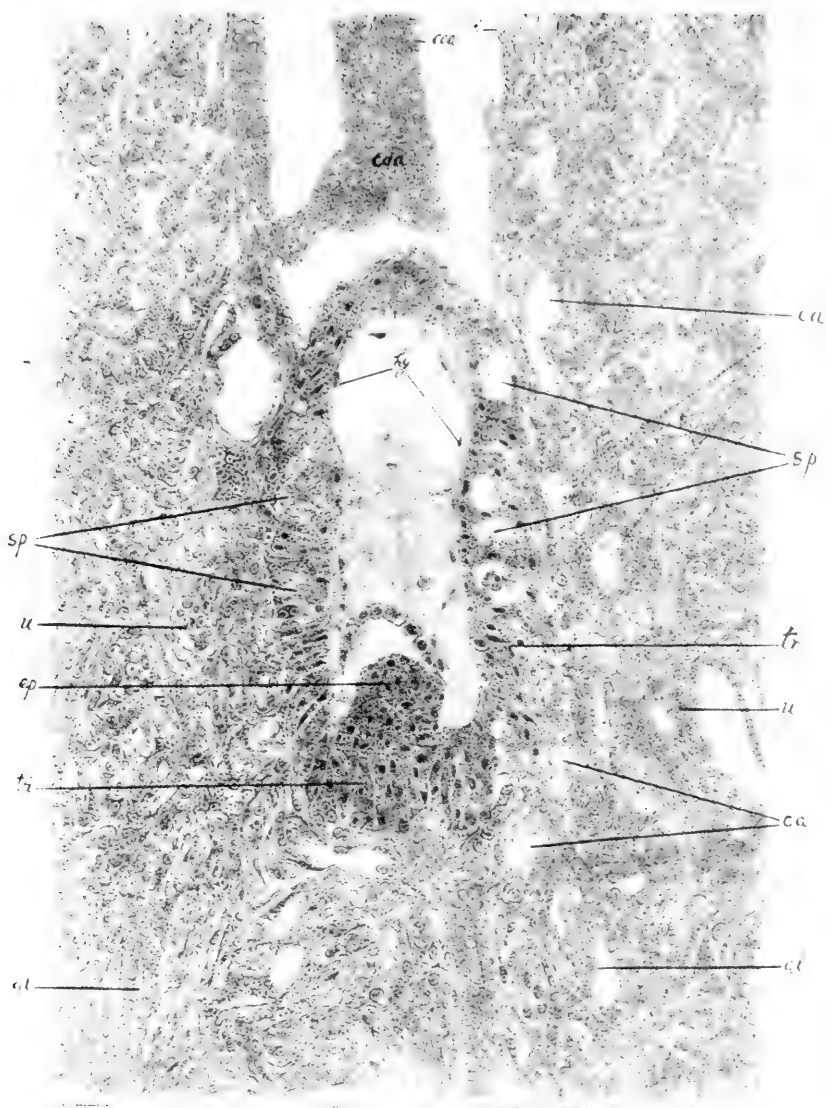


FIG. 1.—Section of an early ovum of a hedgehog embedded in the uterus.

U, uterine wall; *ca*, maternal blood-vessels; *gl*, uterine glands; *tr*, trophoblast; *sp*, lacunae in the trophoblasts; *ep*, trophoblastic mass which is to form the epiblast of the germinal area; *hy*, hypoblast; *coa*, blood clot; *e*, uterine epithelium. (Hubrecht.)

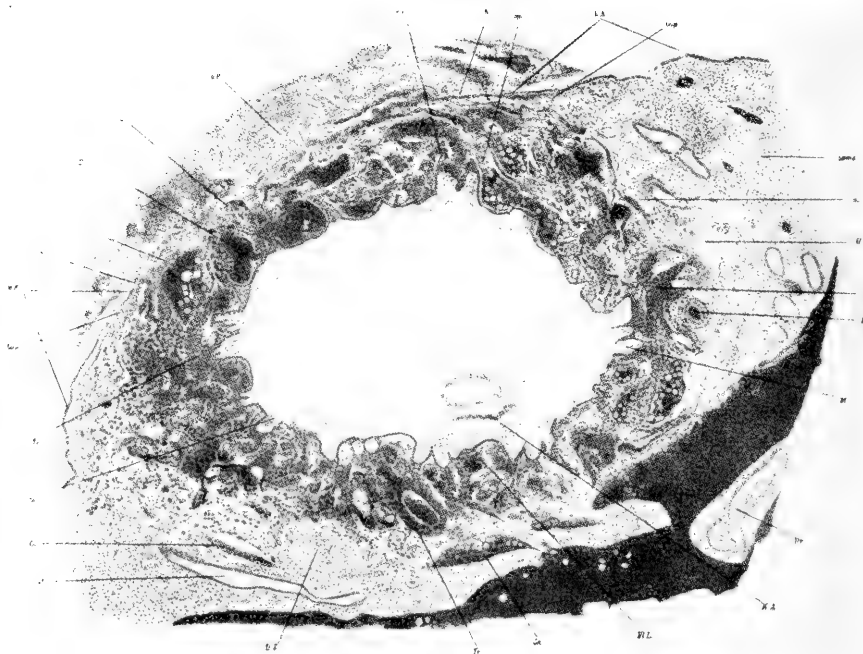


FIG. 2.—Section through ovum embedded in the mucosa. End of first week of pregnancy. Showing the largest diameter of the chorionic vesicle.

G. P., blood clot in the outer polar portion; *a, b*, margins of the opening in the mucosa made by the ovum *U. E.*, uterine epithelium; *ap*, decidua reflexa; *Tr*, trophoblast; *Dr*, uterine glands; *Bl. L.*, lacunæ in the trophoblast containing maternal blood; *K.A.*, embryo; *Comp*, decidua compacta; *M*, fetal mesoblast. (H. Peters.)

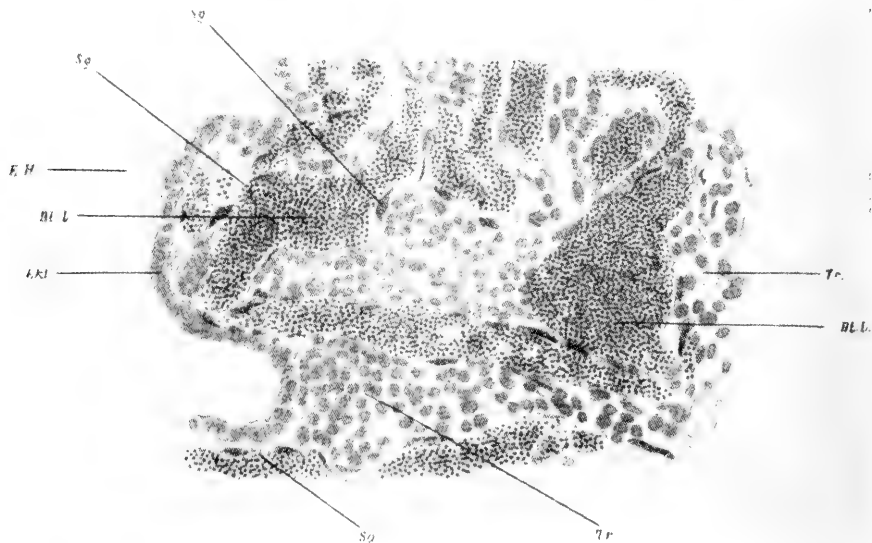


FIG. 3.—Section through the chorionic epiblast layer and part of its trophoblastic extension. First week of pregnancy.

Ek, chorionic epiblast; *Tr*, trophoblast; *Sy*, earliest syncytium; *Bl. L.*, lacunæ in the trophoblast, into which maternal blood has found its way. (H. Peters.)

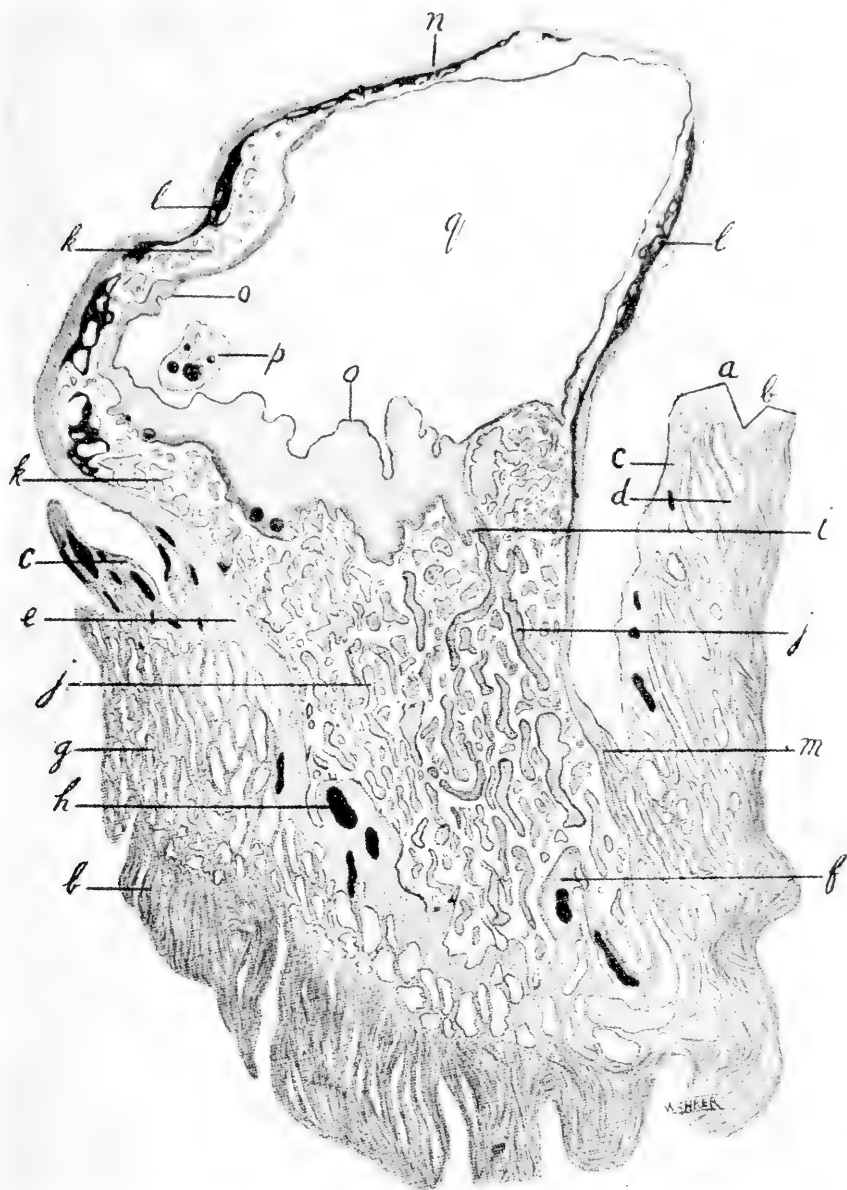


FIG. 4.—Section from uterus in fourth week of pregnancy.

a, decidua vera; *b*, portion of uterine musculature; *c*, compact layer of decidua vera; *d*, spongy layer of vera; *e*, compact layer of serotina; *f*, decidual hillock; *g*, spongy layer of serotina; *h*, blood sinus in serotina; *i*, chorion; *j*, villi of chorion frondosum; *k*, villi of chorion laeve; *l*, fibrin on inner wall of decidua reflexa; *m*, basal portion, and *n*, outer polar portion of reflexa; *o*, amnion; *p*, umbilical cord; *q*, cavity of amnion.

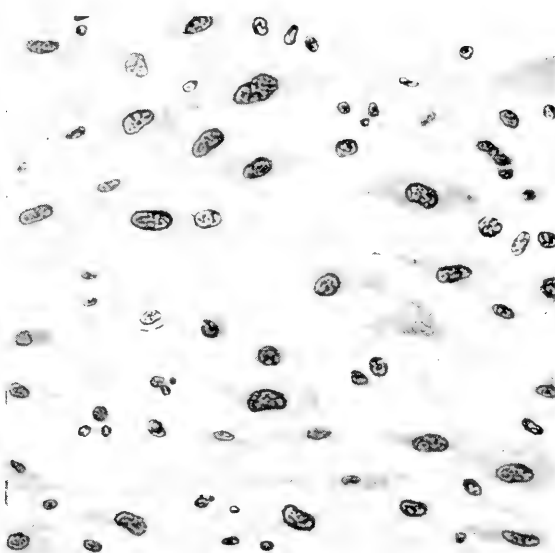


FIG. 5.—Decidua vera in the sixth week of pregnancy. Note the loose structure of the tissue. There are a few leucocytes in the intercellular spaces.



FIG. 6.—Section from the sixth month.

a, decidua cells; *b*, upper margin of decidua serotina; *c*, syncytium showing net-like arrangement.

blastocyst consisted of an outer epiblastic layer (trophosphere) and an inner mass of hypoblastic cells, the latter gradually forming the lining of the primitive gut and the umbilical vesicle. Part of the epiblast forms the embryonic disc. When the mesoblast appears, it divides into an outer somatic and an inner splanchnic layer, the *cœlom* or body cavity being between these. The outer layer, along with the epiblast, forms a double fold which extends over the embryo and blends. The inner portion of the fold forms the amnion. The outer epiblastic layer, together with the subjacent mesoblast, forms the diplotrophoblast.

The splanchnic mesoblast extends around the hypoblast and thus forms the outer covering of the hypoblast canal of the umbilical vesicle. The mesoblast of the latter blends with that of the diplotrophoblast and enters into the formation of a vitelline placenta, the vessels of the villi being derived from the omphalomesenteric. This arrangement is temporary, for owing to the growth of amnion and fetus the umbilical vesicle and its villous projections are gradually detached from the omphalotrophoblast and retrogressive changes take place in them. At the same time the allantoic vessels extend outward and enter into relation with another area of the diplotrophoblast (the so-called allantoidean) to form the permanent placenta.

The early relationships of the blastocyst and uterine mucosa, as demonstrated by Hubrecht, are as follows: The former becomes embedded in the latter, a structure similar to that of the human decidua reflexa being formed. Decidual changes also occur in the mucosa. The trophoblast proliferates markedly, and in it lacunæ appear into which maternal blood finds its way. This early circulation through the trophoblast is established before the above described relationships with the vitelline or allantoic vessels are formed, and indeed is important in contributing to the growth and development of the blastocyst. The stalks of trophoblast between the lacunæ are well termed the pathfinders of the future villi, whether vitelline or allantoic, for the mesoblastic tissue carrying fetal vessels gradually

penetrates them, forming the characteristic vascularized villi of the more advanced stage. Peters' investigation of a human uterus, pregnant only a few days, clearly established that the human blastocyst has the power of embedding itself in the uterine mucosa, the epiblastic cells being able to absorb the epithelium and other elements, and that there is an early proliferation of the epiblast (trophoblast) followed by the formation in it of lacunæ, into which maternal blood finds its way. At the same time the uterine capillaries dilate to form sinuses and the connective tissue cells enlarge to form the so-called decidual cells. As the trophoblastic lacunæ enlarge the trabeculæ between them become smaller and may be regarded as the primitive villi or pathfinders of the permanent villi, which are formed when the mesoblast with fetal vessels penetrates these epiblastic stalks at a later period. The lacunæ are the earliest stages in the formation of the future intervillous spaces.

These changes take place around the entire blastocyst. Later, retrogressive and degenerative changes take place everywhere normally, except at the area serotina, where the permanent placental development continues.

The old views as to the origin of the villi and intervillous spaces as held by Turner, Balfour and others, can no longer be entertained. They thought that the early villous projections of the ovum extended into dilated maternal capillaries and believed that this arrangement was analogous to that in the pig where the villi fit into vascular crypts in the maternal mucosa.

An important observation of Peters was the early transformation of trophoblastic cells lining the lacunar spaces into a plasmodial layer—the syncytium. Though this was not described by Hubrecht in the hedgehog, he has since recognized a similar transformation in various other Insectivora, and it has also been observed in other Mammalian orders. In the human placenta the syncytial covering of the villi has long been known, but until recently had never been correctly described.

Among the Rodentia and Cheiroptera many places of placentation closely resemble those found in the hedgehog.

Among the Lemuridæ the usual form of placentation is analogous to that found in the Ungulates. There is a diffuse villiferous formation which corresponds with depressions in the uterine mucosa, separation taking place very easily at birth. In one variety, *Tarsius Spectrum*, found in the Indian Archipelago, Hubrecht has recently noted that the placentation is not of this character but corresponds to that found in the Insectivora, rodents, bats and man. The blastocyst becomes embedded in the uterine mucosa followed by marked proliferation of trophoblast in which lacunæ are formed. The fully formed placenta is discoid and not diffuse. The umbilical vesicle does not form a primary omphaloidean placenta but remains undeveloped within the cavity of the embryonic vesicle.

It is, therefore, evident that *Tarsius* is very closely related both to the Insectivora and the Primates.

Hitherto, the Lemuridæ have been termed Prosimiæ, being considered an important connecting link between the Primates and lower mammals. This view, in the light of present knowledge both embryological and palæontological, must be considered as wrong. *Tarsius* alone is worthy of such a description, though Hubrecht believes it should be actually classed among the Primates. Certainly, its inclusion among the Lemuridæ is not justified.

As far as investigations of the monkeys have been carried out, there is every reason to believe that the early relationships of blastocyst and uterine mucosa are similar to those found in the Insectivora and man. It is unfortunate that as yet so little has been done in the investigation of placentation both in old and new world forms. Much of the work which has appeared has been marred by misinterpretation. Thus Selenka has pictured sections which bear a close resemblance to those made from specimens of the human pregnant uterus, but he has wrongly described them, inasmuch as he states that the fetal villi dip into mucosal glands and that the layer which is undoubtedly fetal epiblast is derived from uterine glandular and surface epithelium. It is to be hoped that this field of

research will soon be more widely explored. I now desire to direct your attention to certain interesting changes which occur in connection with the growth and development of the human placenta.

Changes in the Uterine Mucosa.—It is impossible to state exactly when the characteristic alterations in the mucosa begin, though Peters' specimen indicates that they are in progress during the very first days of gestation. Hitherto, many observers have accredited to the epithelium of the surface an important part in the formation of the decidua. All careful recent investigations prove that this view is wrong. The surface epithelium and that lining the glands gradually degenerate and to a great extent disappear within the early months. The interglandular connective tissue, on the other hand, shows activity especially during this period. There is hyperplasia and marked hypertrophy of the cellular elements, giving rise to the decidual cells. This transformation always begins nearest the surface of the mucosa, gradually extending outward towards the spongy layer. Connective tissue elements alone undergo this change. Those who have claimed that decidual cells arise from leucocytes or the glandular and surface epithelium are entirely wrong.

The most important vascular change is the marked dilatation of many capillaries in the superficial portion of the mucosa forming blood-sinuses. It has been claimed by some that extensive rupture of capillaries is an important feature of the early changes in the decidua, but careful investigation proves that this is not the case, hæmorrhage taking place to a very slight extent.

Early Relations Between the Blastocyst and Mucosa.—I have already briefly alluded to Peters' specimen of the pregnant uterus, the earliest which has yet been described. In it the blastocyst was almost entirely embedded in the most superficial part of the mucosa.

The surface epithelium of the mucosa had entirely disappeared. Surrounding the blastocyst was a marked proliferation of its epiblast—the trophoblast. It extended irregularly

into the maternal connective tissue and contained many lacunæ into which maternal blood had found its way. The trophoblast cells were quite distinct from one another, where they lined various lacunæ. Here a fusion seemed to exist—the earliest stage in the formation of syncytium being brought about, according to Peters, partly by the pressure of the blood, partly by the influence of the blood-plasma; in some places degenerating blood-corpuscles were blended with the fused cells. In various parts processes of the trophoblast penetrated maternal blood-sinuses, having absorbed the tissue external to them. The circulation of maternal blood in the lacunæ of the trophoblast is brought about in this way, and is the earliest stage in the development of the intervillous circulation of the fully-formed placenta. The trophoblastic strands between the lacunæ are to be considered as the primitive villi.

In the earliest period it is, therefore, evident that the greatest importance must be attached to the trophoblast. It serves to attach the ovum firmly to the uterine mucosa, at the same time absorbing the tissues and fluids of the latter. It establishes a communication between maternal blood-sinuses and the lacunæ which form in its own substance; an important source of nourishment is thus early provided for the blastocyst, before a circulation is established in the latter.

During the second week the chorionic mesoblast begins to penetrate the primitive villi or trophoblastic stalks, and later becomes gradually vascularized by branches for the umbilical vessels. As the trophoblastic lacunæ increase in size the villi become more distinct. The layer of trophoblast forming the wall of the lacunæ next the decidual tissue becomes very thin, and after an early period is represented by strands of syncytium similar to that found on the villi and chorion.

Irregular extensions project into the lacunæ (intervillous blood-space) and extend outward into the substance of the decidua.

Formation of the Decidua Reflexa.—The origin of the decidua reflexa has been the subject of much controversy for many years. Thus it has been held that when the ovum

attaches itself to the mucosa special projections of the latter grow up around it, forming a complete covering. More recently the view has been advanced that after the ovum is fixed to the mucosa the increase of the latter in thickness leads to the investment of the former, that part which extends over and above the ovum forming the reflexa.

Peters' specimen clearly demonstrates that the early blastocyst rapidly embeds itself in the compact layer of the mucosa, probably by the phagocytic action of the trophoblast which excavates both laterally and deeply. The overhanging portion of the mucosa forms the reflexa, the gap through which the blastocyst enters being closed by blood-clot. At first the reflexa is well vascularized and nourished, but as it increases in area degeneration takes place in it very extensively. It has always been taught that the reflexa blends with the vera and that it more or less forms the superficial layer of the latter during advanced gestation. Recent investigations throw doubt on this view. During the third month the reflexa is usually recognized as a thin membrane in close contact with the vera; in the following month it may in some parts be recognizable, but in others it cannot be traced, the villi of the chorion lying against the vera. At this period it is usually easy to distinguish the vera from the reflexa, because the latter is markedly degenerated, presenting a hyaline or fibrinous appearance, whereas there is very little degeneration in the vera.

Changes in the Decidua Serotina.—The most marked stage of development in that part of the decidua in relation to the placenta is found in the second month. Thereafter, while formative changes take place, other factors exercise an important influence. It becomes somewhat thinned by the intrauterine pressure and is also stretched in a direction parallel to the surface. As a result the gland-spaces gradually become compressed and arranged parallel to the surface, the interglandular trabeculae are thinned and in many parts torn across. The decidual cells also tend to be arranged more or less parallel to the surface.

The glandular epithelium becomes degenerated and to a large

extent disappears. The belief held by many, that it is transformed into syncytium, is no longer tenable.

The degeneration may be partly due to the mechanical disturbances of pressure and stretching, but it may also be due to the rapid formation of decidual cells, in the compacta at least, interfering with the distribution of lymph and so affecting the nutrition of the gland epithelium.

In the connective tissue the most characteristic degeneration is coagulation necrosis, leading to the formation of hyaline areas, first noticed in the serotina about the sixth week. It is interesting to note that these hyaline changes occur in the reflexa before they are found in the serotina, while they are not present in the vera throughout the entire course of pregnancy. The explanation of these differences is somewhat uncertain, though an important influence in their production seems the close relationship of the villi. The degeneration usually begins superficially in the decidua near the attachment of the villi, though in some parts this hyaline layer may be derived from maternal blood. Another factor may possibly favor the degeneration, viz., compression and obliteration of lymph spaces and of many capillaries by the marked growth of decidual cells, thus leading to impaired nutrition of the superficial part of the decidua, especially the reflexa.

True fatty degeneration is rarely found except in pathologic conditions.

Disappearance of degenerated parts of the decidua takes place to a considerable extent during gestation, probably through the blood and lymph streams, the agency of leucocytes and the action of the fetal epiblast. *Pari passu* regeneration of the connective tissue elements occurs throughout pregnancy, the relationship between the constructive and destructive processes varying in different areas and in different cases. Thus at the end of gestation, the serotina, in parts, owing to stretching, compression, degeneration and absorption, may have entirely disappeared, the villi being directly related to the uterine musculature. In most parts, however, it is more or less preserved.

The Chorion.—The early covering of the blastocyst and the changes which it undergoes as it becomes embedded in the uterine mucosa have already been described, viz., proliferation of the epiblast to form the trophoblast, lacunar formation in the latter, the transformation of cells of the trophoblast lining the lacunæ to form syncytium. The latter change rapidly advances so that in the second week it is recognized as a very definite layer, covering the entire chorion and the surface of the serotina and reflexa. The remaining cells of the trophoblast subjacent to the syncytium are cubical or rounded in character, with well-marked outlines, lightly staining cell-substance and round or oval nuclei, forming the so-called Langhans layer. The syncytium consists of darkly staining nucleated granular protoplasm, without cell-boundaries. It varies in thickness, and at intervals projections extend from the surface into the intervillous spaces. This layer seems to serve as a kind of endothelium for the intervillous spaces. It probably aids in preventing coagulation of the circulating maternal blood, and may play an important part in the physiologic processes associated with the interchange between the maternal and fetal blood streams.

While these changes have been taking place, the chorionic mesoblast has been increasing at a rapid rate, forming a central core for the primitive villi as well as those which develop in enormous numbers after the first week. The earliest villi are very simple and run a straight or wavy course from the chorion to the decidua; they gradually become branched, and as they grow the connective-tissue core, with its blood-vessels, becomes larger. Most villi are attached to the decidua by a thick mass of Langhans cells, a few are attached by syncytium.

This description of the chorion applies to the entire blastocyst in the early weeks of gestation. Very soon a differentiation must be made between the placental portion—*chorion frondosum*—and the non-placental part—*chorion læve*.

The changes in the latter need not here be noted in detail. It is sufficient to state that as the reflexa degenerates and thins, the villi in relation to it also degenerate, the epithelium dis-

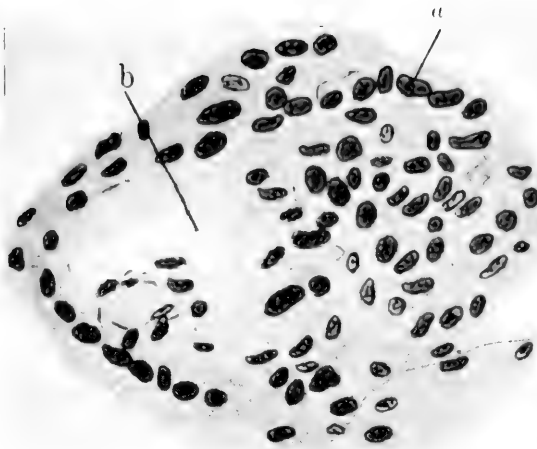


FIG. 7.—Bud of syncytium from intervillous space.
a, nuclei of syncytial cells; *b*, vacuolization in the syncytium.

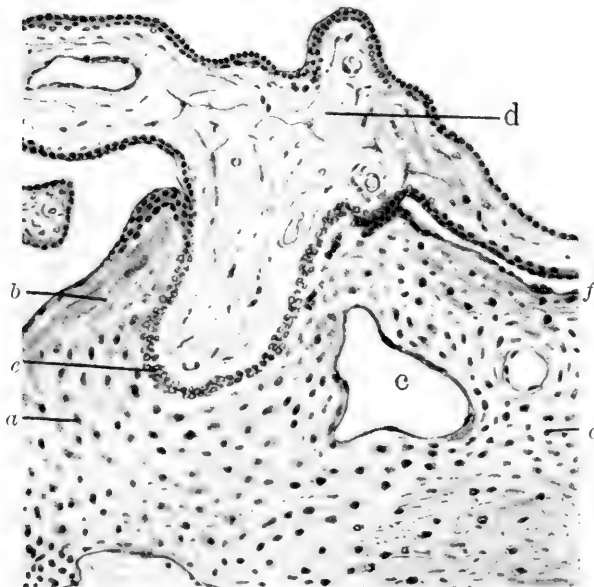


FIG. 8.—Chorionic villus and decidua serotina. Section from the sixth month.
a, decidua cells; *b*, fibrin degeneration; *c*, blood sinus; *d*, villus partially embedded in the decidua; *e*, proliferation of Langhans layer; *f*, upper margin of decidua.

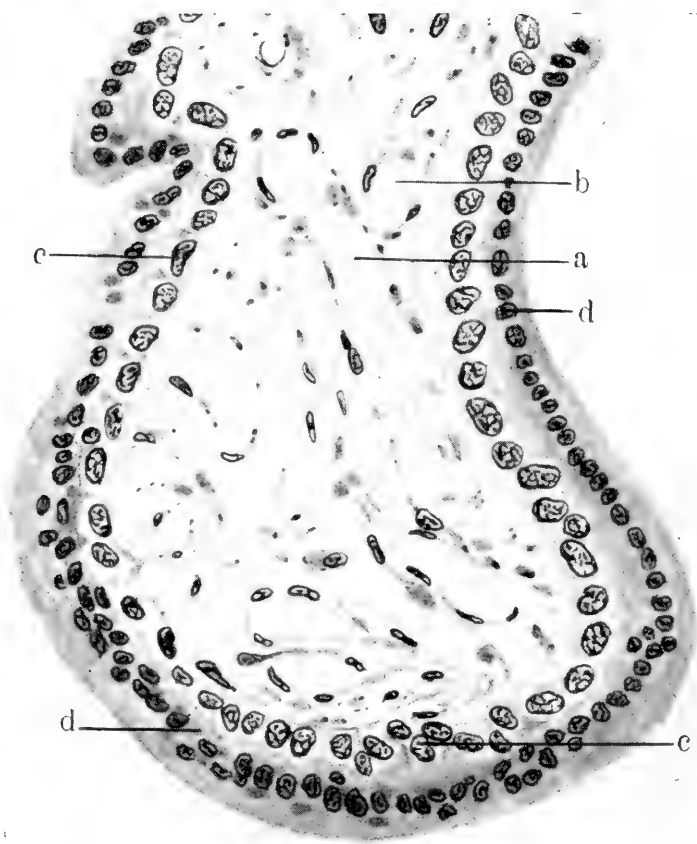


FIG. 9.—Section of chorionic villus at the fifth week.

a, connective-tissue stroma; *b*, capillary; *c*, well-marked layer of Langhans; *d*, syncytium.

appearing and the connective tissue undergoing hyaline changes.

The rest of the chorion related to the serotina continues its development, forming the placenta. Variations in the shape of the latter are due to differences in development and degeneration of villi, in the early weeks of gestation in most cases being probably associated with abnormal development of the reflexal chorion next the serotina.

In the development of the placental chorion there is merely a continuance of the changes already described. The villi become more numerous, longer and more branched, the proliferative activity of the epiblast, however, diminishing markedly after the early months.

By the beginning of the third month the Langhans layer consists of a single or double row of cells, being thinner on the average than the layer of syncytium. As pregnancy advances these layers gradually become thinner, the syncytium undergoing hyaline degeneration, vacuolization and splitting parallel with the surface of the villi; the connective tissue becomes denser and more fibrillated. At the end of pregnancy the Langhans layer of the villi is very poorly marked, in many places consisting of widely separated cells, while the syncytium is scanty and degenerated. In parts both of these layers are entirely absent, the connective tissue being in contact with maternal blood or covered with a layer of fibrin. At the site of attachment of the villi to the decidua the Langhans cells have in most cases disappeared, so that the connective tissue of the villi is in direct contact with the decidual tissue. The nature of the layer covering the villi has long been the subject of marked difference of opinion. Kölliker, many years ago, held that the chorion and villi received a covering of decidual tissue. Ercolani believed that it was derived from the connective tissue, Turner that it was derived from the mucosal epithelium, and Waldeyer that it was an extension of maternal endothelium. Leopold and Eden hold that the Langhans cells are fetal mesoblast. Ercolani, Turner and Kölliker state that the covering of the villi is a single layer. Langhans believes

that it is a double layer, the inner being fetal mesoblast, the outer fetal epiblast. Winkler states that it is double, the inner layer being fetal epiblast and the outer maternal endothelium. Tafani and Romiti believe that there is an inner layer derived from maternal connective tissue and an outer from maternal endothelium. Jassinski states that the inner layer is fetal epiblast and the outer maternal epithelium derived from the mucosal glands. Keibel describes three layers, the two inner being fetal and the outer maternal endothelium. Schroder von der Kolk says that there are three layers derived from maternal decidual tissue.

Such differences are to be explained by the defective character of the investigations on which they were based. Generalizations have been made from the study of single specimens without reference to other periods of pregnancy. In recent years very careful studies have been made of specimens of the pregnant uterus from every month of gestation, and in this way it has been clearly ascertained that the chorion and villi are covered only with derivations of the fetal epiblast and never with maternal tissue of any kind. The maternal epithelium covering the mucosa is destroyed by the trophoblast as the blastocyst burrows into it. The epithelium lining the mucosal glands degenerates and to a great extent disappears as pregnancy advances. The maternal endothelium shows no proliferative activity nor any tendency to extend outward. In the early stages of pregnancy the trophoblastic strands in extending outwards may occasionally lie immediately under the endothelial layer of a maternal sinus, and this might be misinterpreted as an extension of endothelium over the trophoblast. At all periods a thin layer of syncytium may bear some resemblance to an endothelial layer, especially in the advanced months when the syncytium tends to be split parallel with the surface of the villus. The derivation of syncytium from the early trophoblast has been established beyond any doubt. Besides being found on the chorion it occurs on the surface of the serotina between the villi, being left there after the original trophoblast lacunæ have increased

to form the large intervillous spaces. It is found in the substance of the decidua serotina, either detached from the surface or continuous with syncytium on the latter; it may even be found in the muscular part of the wall. Extensions into maternal blood-sinuses and gland-spaces may be traced. Small portions are undoubtedly transported by the maternal blood-stream throughout gestation. Syncytium extends into the reflexa to a very slight extent in the early months. It is not found in the decidua vera.

Relation of the Intervillous Circulation to the Blood-vessels of the Mucosa.—For many years the formation of the intervillous spaces has been a puzzle to investigators, and it is only recently that it has been clearly understood. Peters' early specimen afforded the earliest stage, and it proved to be what had been predicted by Hubrecht, as the result of his study of placentation in the hedgehog. The development of lacunæ in the early trophoblast is followed by the entrance of maternal blood into them. The capillaries of the mucosa nearest the blastocyst rapidly enlarge while the latter is becoming embedded, forming large sinuses. These are opened by the phagocytic action of the trophoblast, the blood finding its way into the lacunæ, which gradually communicate and form a system of canals throughout the entire trophoblast. The maternal blood is thus brought early into relationship with the extensive epiblastic covering of the chorion. As gestation advances, this circulation is restricted to that portion of the chorion attached to the serotina. In some cases it may persist in a neighboring part attached to the reflexa, giving rise to a more or less well-marked reflexal placenta.

As the lacunæ enlarge and the villi become more elongated and slender, the relationship between the two is altered. Whereas at first the maternal blood flows in channels among the primitive villi, in later stages the villi may be described as hanging in one enormous blood-space, which lies between the decidua serotina and the chorion. Yet it must be clearly understood that this blood-space is only a magnification of the early canal-system. In the early days the lacunæ are through-

out lined by epiblast, but later owing to the disappearance of this tissue, excepting small portions of syncytium on the surface of the decidua, the boundary of the large intervillous space on the maternal side is largely the connective tissue of the decidua serotina. The villi which are attached to the latter are mostly fastened at their ends. The old view that they were suspended in enlarged vessels of the mucosa must be entirely abandoned. It is very rare to find a villus hanging into the open mouth of a maternal sinus at the surface of the decidua or attached to its wall. Neither is there any truth in the statement that maternal endothelium extends outward so as to cover the villi. The layer long described as endothelium is really a thin syncytial investment derived from chorionic epiblast.

Regarding the maternal blood-supply to the intervillous space, it is widely believed that definite arteries and veins communicate with it. This is a wrong description. It is rare that a true artery or vein can be found in the superficial part of the mucosa. The vessels in this area are mostly capillaries, though a few may be found consisting of an endothelial tube surrounded by one or two layers of flattened connective-tissue cells. The sinuses which communicate with the intervillous space are enlarged capillaries. The opening will direct an afferent or efferent current according to whether it is near the arterial or venous end of the sinus. Such an arrangement ensures a steady flow of blood among the villi. If arteries opened directly into the intervillous space, the jets of blood might be a source of danger to the villi, tearing them and separating them from their attachments. The tortuous course of the arteries in the muscular part of the wall and the deeper portion of the mucosa further safeguards the circulation among the villi, for it necessitates a diminution of the force with which the blood stream is moved onward. The enlargement of the capillaries lessens it to a greater extent. The disposition of the veins is such as to favor the removal of the deoxygenated blood from the intervillous space; they are not tortuous like the arteries but run a straighter course.



FIG. 10.—Section from sixth week pregnant uterus.

a, fibrinous degeneration in the serotina; *b*, decidual hillock; *c*, proliferation of Langhans cells uniting a villus with a decidual hillock; *d*, syncytium lining the upper margin of the decidua; *e*, connective tissue core of a villus.

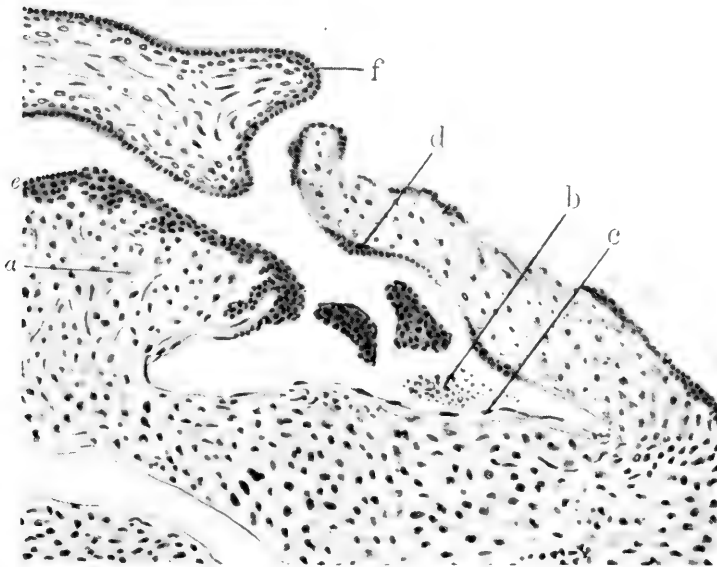


FIG. 11.—Decidua serotina and chorionic villi at the sixth week.

a, decidual cells; *b*, maternal blood sinus opening into the placental blood spaces; *c*, endothelial lining of the blood sinus; *d*, syncytium adhering to the wall of the blood-vessel; *e*, syncytium covering the upper margin of the decidua; *f*, chorionic villus.

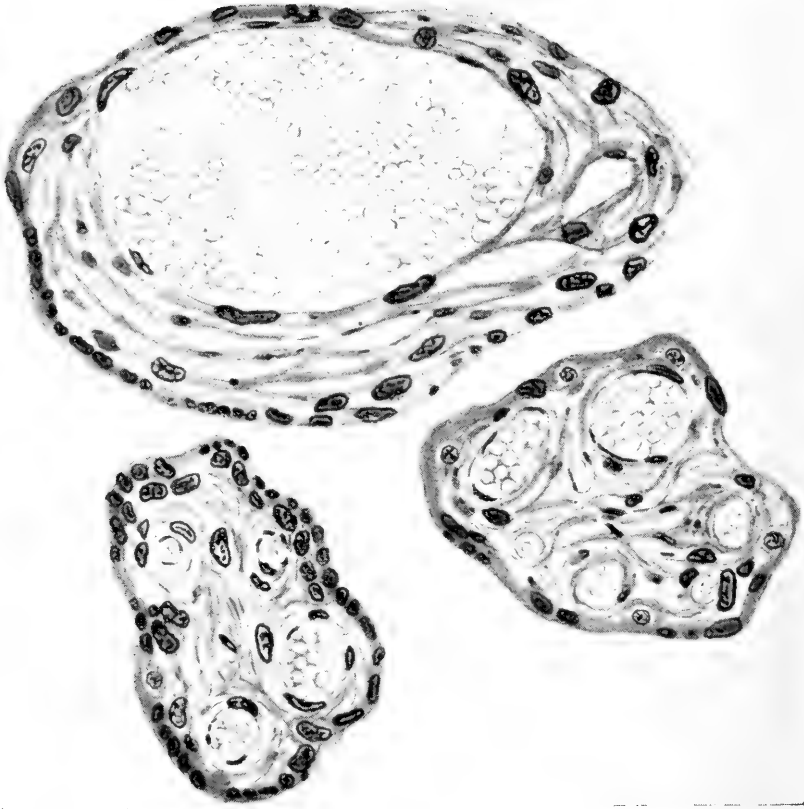


FIG. 12.—Sections of full-term chorionic villi. Compare the thickness of the epithelium covering of the villi with sections in the early months. Note also the size of the blood-vessels.

The entire arrangement is of such a nature as to render the intervillous blood largely independent of sudden changes in the maternal vascular system. It is not a swift-flowing, pulsating current, but a steadily-moving mass of blood.

SUMMARY

1. Comparative studies show that placentation in the human female is very similar to that found in the highest monkeys. From the close resemblances to the placentation in the Insectivora strong support is given to the view of Huxley, who taught that this order holds a central position among the higher Mammalia and is to be regarded as having been represented among the most primitive monodelphian types.

2. The old view that human placentation is related directly to that found in the Ungulata and Carnivora is now thoroughly discredited.

3. In most of the orders of Monodelphian mammals there is only one placenta, viz., that derived from chorionic tissue vascularized from fetal allantoic vessels.

4. In three orders, Rodentia, Insectivora, Cheiroptera, there is as well a preliminary temporary placenta derived from a part of the chorion which becomes vascularized by the vitelline vessels of the yolk-sac.

5. In the human female the vitelline vessels may sometimes form the permanent supply to the villi of the placenta, viz., in cases of malformed fetus, in which the hypogastric arteries, from which the umbilical are ordinarily derived, are not developed.

6. The term "allantoic," as applied to the human placenta, should not be used. The allantois does not enter into its formation, nor does it give rise to the link which binds the fetus to the chorion. The latter is never separated from the former. When the embryo is differentiated it remains attached to the chorion by a stalk afterwards known as the "abdominal stalk." Through this the hypogastric arteries send branches to the chorion and its villous extensions.

7. In the early ovum there is a proliferation of the epiblastic layer covering the blastocyst, known as the trophoblast.

8. The blastocyst becomes rapidly embedded in the superficial layer of the uterine mucosa by a process of burrowing, the trophoblastic cells possessing a phagocytic action. Strands of these cells extend outward into the maternal tissue in various directions.

9. *Pari passu* with the embedding of the blastocyst there occur marked changes in the mucosa, viz., degeneration in the epithelium on the surface and in the glands, hypertrophy and hyperplasia of the interglandular cellular elements and capillary enlargement. The altered mucosa is known as the decidua.

10. These changes in the mucosa are most marked immediately under the embedded blastocyst.

11. In the very earliest stage, the blastocyst absorbs nourishment

from the fluids of the mucosa as well as from the solids destroyed by the trophoblastic cells.

12. While in process of embedding, vacuolization occurs in many portions of the trophoblast, forming lacunæ of irregular shapes and sizes. In the surrounding maternal tissues many capillaries dilate to form large blood-sinuses.

13. The walls of the latter are opened by the phagocytic action of the trophoblast at many points. A direct communication is thereby formed between the maternal vessels and the trophoblastic lacunæ. As the latter enlarge and communicate with one another an intra-trophoblastic lacunar circulation is thus established, being practically an extension of the maternal circulation. From it nourishment is derived by the ovum.

14. As the trophoblastic lacunæ enlarge, the tissue between them becomes reduced to the condition of irregularly shaped trabeculæ, which extend from the outer layer of the ovum, henceforth known as the chorion, to the maternal tissue. These are to be regarded as the earliest or primitive villi, the pathfinders for the permanent villi. At first they are entirely epiblastic cells. Gradually, they are penetrated by extensions of the mesoblast of the ovum, which thus form a central core in each. In the latter gradually develop terminal capillary loops derived from the umbilical vessels of the fetus. As development proceeds the mesoblastic core increases and forms the main portion of the villus, while the epiblastic cells covering it are reduced to a thin layer.

15. The cells of the latter, bathed by the circulating maternal blood, begin to be transformed within the first week into a continuous layer of plasmodial character, the outlines of the cells being lost, though the nuclei remain very prominent. This layer is termed *syncytium*. The unaltered subjacent epiblastic cells are known as the Langhans layer.

16. Neither the epithelium, endothelium nor connective tissue of the uterine mucosa in any way contributes to the formation of the syncytium. The maternal blood, however, influences its formation.

17. The enlarged trophoblastic lacunar blood-system is known, after the formation of villi, as the intervillous blood-space (or spaces). At first it is found around the greater part of the ovum. Later, its development is usually restricted to a circumscribed area, viz., where the outermost portion of the ovum is applied to the uterine mucosa.

18. It is at this area that there occurs the enormous outgrowth of villous extensions of the chorion. Many of them extend across the intervillous space and become attached to the maternal tissue: others hang free in the circulating blood. The large mass formed by the chorionic villous development is known as the placenta. No maternal tissue enters into its formation.

19. The villi do not burrow deeply into the decidua. They are attached to the latter by a proliferation of their epiblastic covering. Detached portions of syncytium are found in all parts of the mucosa and may often be traced in the musculature of the uterine wall. They

as well as small portions of the villi, may be transported by the maternal vessels to distant parts.

20. The superficial portion of the mucosa, which overhangs the embedded ovum, closes over the latter and forms the decidua reflexa.

After the early days the chorionic development in relation to it is very much less marked than that which occurs in relation to the deeper portion of the mucosa, and it usually ceases altogether in a few weeks. Occasionally, it continues to advance, giving rise to a well-formed placental arrangement. When the latter is in the lower portion of the uterus the condition is known as *placenta prævia*.

THE PARASITISM OF THE TUBERCLE BACILLUS AND ITS BEARING ON INFECTION AND IMMUNITY*

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THE present-day problems in tuberculosis which can be approached by experimental or at least by laboratory methods manifest themselves in three different ways:

1. In the somewhat chaotic condition of opinion concerning the avenues through which tubercle bacilli gain a foothold in the body.

2. In the wide divergence of opinion concerning the relation of bovine to human tuberculosis; and

3. In the general trend of studies toward the problem of specific immunity, with special reference to prevention and treatment.

These three problems, though distinct, are interrelated, and in a lecture of this kind, in which some freedom in the statement of theories and hypotheses and a rather broad treatment of the subject are not only permissible but desirable, it must be necessary to deal with each, to some extent at least. The most important of the three is the one dealing with immunity, and my statements will be grouped around and directed toward it as a focal point.

The method of treating the subject will be from a biologic standpoint which assumes as a basis for discussion a complex relationship established in time by a selective adaptation between two living organisms, of which one is a parasite of the other. Whatever pathologic processes of constant character are the expression of this parasitism, such as tubercle forma-

* Lecture delivered March 10, 1906.

tion, for example, are regarded as the result of an interaction of two organisms rather than the work of one alone.

Viewed from this standpoint, this tendency toward a state of equilibrium between host and parasite is disturbed by any change of condition which influences either parasite or host. It varies with the species, race, nationality or even family of the host and many other accessory conditions. It depends on the race of tubercle bacilli. In experiments such conditions as age of culture, total period of cultivation, character of the culture medium, condition of aggregation of the bacilli, mode of application and dosage are of great importance in determining the outcome of the experiment. Similarly the outcome will vary according to the species of animal on which we are experimenting.

Much of the experimental inquiry of the past has been along too narrow lines and with the conditions too poorly defined. We have lost sight of the general relations of bacteria to animal life. Our haste to take the animal and bacterial mechanisms to pieces and to test the individual tissues and components has crowded out the broader view that the host fights more as a unit. We had almost forgotten to take into consideration the flexibility and adaptability of the micro-organisms themselves.

In a recent lecture I presented in a somewhat new aspect the relationship between host and parasite by pointing out that in a stable parasitism the parasite is in command of a mode of exit from the body as well as one of entry. Both are necessary for the continued existence of the parasite as such. The evolution of this process has brought with it two related conditions: first, a lowered virulence or invasive power of the microbe; and, second, the tendency to attack mucous membranes, cutaneous surfaces or organs in direct communication with the exterior. The lowered virulence is only another expression for localization on the external surfaces among which mucous membranes and the respiratory tract may be placed for present purposes. As a result of this adaptation tuberculosis has taken largely the form of phthisis. That is,

the parasite has become localized in an organ in direct communication with the exterior, yet largely protected from miscellaneous bacterial and other parasites. Its modes of exit and of entry are identical. The bacillus may vegetate in the lung tissue and it may be easily discharged outwardly.

Phthisis, however, is only one, even if the preponderating type, of tuberculosis. Much has been made of the other manifestations. Among these, disease of the lymph nodes, bones, kidney, brain and spinal cord must be considered aberrant from the standpoint of the bacillus, for in these situations, with the possible exception of tuberculosis of the kidneys, the bacillus is doomed to an ignominious destruction, because there is no exit. These aberrant forms of parasitism are most frequent in childhood, because, perhaps, the tubercle bacillus being, as it were, keyed to adult life, is for that reason more invasive for childhood.

To the biologist these types of disease are of interest as suggesting problems in susceptibility and resistance which, as stated above, are the problems through which experimental medicine may deal practically with this disease.

In order to bring out in relief certain general biologic phenomena of tuberculosis, I shall discuss, first, very briefly the mode of invasion of the body by the tubercle bacillus, then sketch a theory of the interaction of the body and the bacillus. I shall then discuss the tuberculin reaction and the action of dead tubercle bacilli and the procedures suggested or used for producing an increased resistance of the body.

I. THE INVASION OF THE BODY BY THE TUBERCLE BACILLUS.

Bearing on immunity, the problem which deals with the primary seat of tuberculosis and its relation to the portal of entry deserves consideration, since it is, to a certain degree, an index of susceptibility. The theories concerned with the mode of invasion of the tubercle bacillus may be classed under four heads:

1. The inhalation of dried sputa, as laid down by Koch and elaborated by Cornet and others.

2. The inhalation of moist particles, or spray infection, as formulated and worked out by Flügge and his pupils.

3. Congenital tuberculosis, resulting from infection *in utero*, as defended by Baumgarten.

4. The infection through milk in infancy associated with a greater or lesser degree of latency until puberty and even later, the theory recently championed by Behring.

In taking some definite stand as to which of these theories, if not all, should have our support, we may gain some evidence from a study of the primary seat of the disease, *i.e.*, that place in the body where the presence of the bacillus is shown either by the existence of actual lesions or by animal inoculations.

In the largest number of cases of tuberculosis the lungs themselves have been regarded as the primary seat of the multiplying bacilli. In children, however, other conditions frequently prevail, and the primary seat of the active process may be in the cervical, the bronchial and the mesenteric lymph nodes; many authors have called attention to this fact. Weigert¹ referred to this over twenty years ago.

The most recent monograph of Harbitz² refers to it as follows: "The general rule in cases of children is that the lymph nodes are primarily attacked and that the lungs are infected from them. General experience teaches that isolated tuberculosis of bronchial nodes is quite common while isolated pulmonary tuberculosis, with or without a slight and plainly secondary lymph node tuberculosis, is a rarity in children."

Ribbert goes so far as to assume that pulmonary tuberculosis is mainly hematogenous in origin, the source of the infection being some lymph node primarily diseased.

¹ Deutsche med. Wochft., 1903, p. 735. "In adults, for instance, we find so frequently the familiar tuberculous lymphatics issuing from ulcerations in the intestines while the lymph glands pertaining to them show comparatively slight changes. In children, on the contrary, we often encounter the reverse, pronounced caseous changes and swelling in the mesenteric glands while no morbid process can be detected in the afferent lymphatics, even in their trunk region." He refers also to similar conditions for the bronchi, the mouth and the skin.

² Jour. Inf. Diseases II, 1905, p. 143.

Petruschky, in his various publications dealing with the curative power of tuberculin, has identified himself so thoroughly with this view as to regard and to classify lymph-node tuberculosis as the first stage in tuberculosis generally.

Baumgarten³ has contended and still contends that tubercle bacilli always produce some lesion at the point of entry into the body. In taking this position he relies on animal experiments; but there are objections to animal experiments, inherent in the difficulty of approximating natural conditions. The local lesion in animals may be due to a variety of causes, among which are local trauma, dead and attenuated bacilli and chemotactic substances due to autolysis in the cultures, and the want of adaptation of the bacillus to the species of animal used. Compare these with the entry of a solitary bacillus or perhaps several bacilli in a dried condition, without producing trauma or any chemotactic response, and we see at once the difference between the natural and the experimental mode of invasion.

The invasion of tubercle bacilli into the lymph nodes without causing disease at the point of entry has interested me since I began the study of bovine tuberculosis, in which disease such invasion is the rule, and I shall take this opportunity, therefore, of describing this phenomenon in the cattle disease more in detail. I will premise my remarks on this subject by stating that mammalian tuberculosis appears in two independent types—human and bovine. If either human or bovine type were suppressed the other would still continue. The best evidence on this point was presented by Kitasato, who demonstrated that in Japan the human disease existed in its usual activity, though the cattle disease was absent and milk formed no appreciable element in the food of children. Most other mammals at times have been found infected, either from human or bovine sources. In these a satisfactory mechanism does not exist to perpetuate a porcine or canine or feline type of disease. The bovine disease is the best, therefore, for study, next to that of man himself.

³ Berliner klin. Wochft., 1905, p. 1329.

Soon after the recognition of tuberculin as a valuable diagnostic agent, astonishment and consternation were created by the discovery that a very large percentage of the best dairy cattle of the world reacted to tuberculin. Under the influence of this discovery some ill-considered laws were passed to destroy all reacting cows and their flesh, for the purpose of eradicating the disease. During these few years of active warfare, beginning in 1893, I was able to make autopsies on about 350 head of cattle which had reacted to tuberculin. This enabled me to get a good composite picture, as it were, of the early stages of the disease and to determine the primary foci with considerable accuracy. A portion of the results of this investigation was published in 1894⁴; the rest has remained in manuscript form.

In cattle, tuberculosis is an exquisitely parasitic disease, in which the chief seat of the lesions is in the lymph nodes. Next in order come the lungs, then the liver and serous membranes. Furthermore, it may occasionally be encountered in almost any other organ and tissue.

There are three portals of entry, the upper respiratory tract (mouth and nose), the lungs themselves and the small intestines. Very rarely the skin or subcutis has given entrance. The infection through these portals is indicated chiefly by disease of the corresponding lymph nodes. In the head, for example, the pair of retropharyngeal glands are the chief indices of infection. They are situated close together under the mucous membrane covering the dorsal posterior wall of the nasopharynx. The other lymph-nodes of the head are infrequently diseased and need not be considered here. The mucous membrane is free of disease; the tonsils are very rarely infected. The progress of the infection along the chain of nodes in the neck is slow, and the infection of the head glands has little, if anything, to do with the primary or secondary disease in the thorax.

Tuberculosis due to inhalation of tubercle bacilli is by far

⁴ Bulletin No. 7, 1894, Bureau. An. Ind. U. S. Dept. Agric.

the most common. The lungs and associated lymph nodes may be infected or only the latter. The thoracic lymph nodes in cattle belong to three systems, the tracheal and bronchial nodes, closely attached to the trachea and its branches, and draining the peribronchial and perivascular lymphatics, the dorsal mediastinal chain or chains dorsal of and resting on the pillars of the diaphragm and on the esophagus, which probably drain the lymphatics of the lung tissue itself, and the anterior mediastinal glands situated under the first rib; that is to say, in the apex of the thorax. That the bronchial and dorsal mediastinal glands drain the lungs is shown by their similar structure, pigmentation and contents of very fine particles of mineral matter coming from the air. The third group has no pigment and probably drains the pleural cavities only. It may also stand in some relation to the cervical nodes.

In all herds which were examined there was a considerable number of animals in which the pulmonary infection resulted in lymph-node disease only. In one herd of sixty animals, for example, of which fifty-three were infected, twenty-seven had tuberculosis of the thoracic lymph nodes, but no lesions in the lungs.

Next in frequency comes disease of the lungs themselves. The chief seat is in the large caudal lobes. In man the upper or cephalic lobes are the preferred seat. In cattle the invasion is just where one might suppose it to be when coming from bacilli suspended in air; it is in the direct line of the current and in the lobe which goes through the widest excursions. That most of the infection lodges here I also infer from the fact that the one mediastinal gland which evidently drains this portion of the lungs is the most frequently infected lymph node in the whole body. The infection through the intestines shows itself exclusively in tuberculosis of the mesenteric lymph nodes and in disease of the liver. Lesions of the mucous membrane are extremely rare.

This very hasty and imperfect sketch of the primary foci of tuberculosis in cattle shows that the bacilli usually enter the system, first, in the inspired air or in the food through

the mucosa of the mouth or throat; secondly, through the lungs in the inspired air, and, thirdly, through the intestinal mucosa in bacilli swallowed in the food. The most striking fact is the passage of the bacilli through the mucous membrane or the air cells into the associated lymph nodes without leaving any trace visible to the naked eye or detected by manipulation. I am convinced, therefore, that Baumgarten's theory can not be maintained in the bovine disease and that tubercle bacilli may pass through at least one gateway of the body without being detained.

The tendency of the tubercle bacillus to settle down and to multiply in the lymph nodes in cattle is manifested in still another way. When the disease becomes generalized by the escape of bacilli from some primary focus into the general circulation, the secondary disease does not give rise to a miliary tuberculosis, but isolated foci may appear in various organs. Even these may be absent and the infection of the organ or the passage of bacilli through it indicated by marked affection of the corresponding lymph glands. Thus the evidence that bacilli have passed through the liver and kidneys is frequently indicated only by tuberculous portal and renal lymph nodes respectively. Evidence of udder infection is frequently presented only by tuberculous pubic lymph nodes. That submiliary tubercles may be found in these organs is not to be denied. I have found a few in the liver in an advanced stage of the disease, composed only of a giant cell and a few epithelioid cells around it. The fact remains that the lymph nodes act toward these organs very much as the lymph nodes of the lungs do in the primary infection.

There are a few other data derived from a study of the distribution of tuberculous lesions in cattle which are of interest here. In the disintegration of pulmonary foci the bacilli may pass in two directions, into the associated lymph node or outward by rupture into the air tubes, or both ways at the same time. Passage into the lymph channels is signalized by an enormous hyperplasia of the dorsal mediastinal and certain bronchial nodes. The bulk of these may be in-

creased from twenty to thirty times. The tuberculous process is in the same stage throughout, which indicates a sudden flooding of the gland. When the discharge is outward, yellowish, caseous masses are found at the autopsy in the smaller air tubes. Ravenel has demonstrated that these masses are actually ejected during coughing. The mucosa of the air tubes themselves is not infected primarily, and eruptions, ulcers and catarrh are subsequent to the discharge of caseous matter. The latter acts both as an irritant and an infecting substance. Infection of other, notably the cephalic or smaller, lobes is brought about by aspiration of the caseous masses. These smaller lobes are more dependent and subject to the gravitation of fluids and semisolid matter.

The careful noting at the autopsy of the approximate age of the tuberculous lesions led me to conclude that infection through one of the avenues mentioned has, as a rule, nothing to do with the others. That is, there seemed to be no connection between tuberculosis of the mesenteric glands and pulmonary disease. It was noticed, however, that the stage of disease in the thoracic and abdominal lymph nodes was in many cases the same. The inference was that the animal was infected at the same time through two or even three different portals. The theory of Behring that tuberculosis starts in early life through the digestive tract is inapplicable as a rule to the bovine disease.

Concerning the mode of invasion of tubercle bacilli in rabbits and guinea-pigs through the natural portals, without the infliction of a trauma, as by subcutaneous inoculation, or the circumventing of certain channels, as by injections directly into the peritoneal cavity or the blood, I have no data of my own. There is evidence, however, to show that in these animals also the lymph nodes form the earliest foci of multiplication in feeding and inhalation experiments, and that the bacilli soon break away through this barrier and are diffused in the blood current over the body.

In the pig the ingestion of infected milk leads at first to tuberculosis of the head and cervical lymph nodes and those

of the mesentery from which stations generalized infection by means of the blood takes place very soon.

A most important question is raised by this penetration of the tubercle bacilli to lymph nodes. How far may they penetrate before they settle down? Do they go beyond the first lymph node? Do they ever reach the blood directly from without? The conservative notions of twelve years ago would hardly admit the penetration of tubercle bacilli below the mucous membranes. To-day the extreme and radical notion of Behring that infection occurs early in life and may remain latent and that tuberculosis in later life largely dates from infancy is being seriously and widely discussed. This preparedness to receive and to discuss such a statement is partly due to the strides made in the study of parasitism. We have become accustomed to the complicated dual-host system of malarial and other blood parasites, the wanderings of the larvæ of *uncinaria* from skin to duodenum and larvæ of certain flies from oesophagus to skin. The revival of instructive studies in animal parasites brings back again the complicated life cycle of tape-worms and flukes. Among the bacteria it seems well established that glanders bacilli may enter the body of horses through the digestive tract. Nicolas and Descos⁵ showed that in the dog tubercle bacilli may appear in the thoracic duct after a meal of fatty substances impregnated with them. Ravenel⁶ confirmed their observations. There is good reason, then, for anticipating discoveries or theories which might greatly simplify our view of infection.

In my studies of the bovine disease I was unable to see anything more than the localization in the lymph nodes of the invaded part or perhaps a very slow creeping along to the succeeding nodes. I am not inclined to accept the extreme view that tubercle bacilli may penetrate very far into the system at the start. The view that they may enter the blood during invasion is derived partly from artificial inoculations.

⁵ Jour. de Physiol. et de Path. Gén., vol. iv, 1902, p. 910.

⁶ Jour. Med. Research, vol. x, 1903, p. 460.

In such experiments more or less injury is always inflicted and the bacilli may enter both blood vessels and lymphatics. In the spontaneous infection, the bacilli enter the lymphatics only and the nodes act as a temporary or permanent barrier. The positive experiments with dogs quoted above can not very well be generalized to apply to the spontaneous disease until similar experiments have been made on other species with bacilli from various sources.

II. THE RECIPROCAL ACTION OF BACILLI AND THE INVADDED ANIMAL TISSUES.

The passage of tubercle bacilli through mucous membranes and the alveoli of the lungs into the nearest lymph nodes is probably made in the same way and by means of the same agencies by which particles of soot, quartz and other mineral particles are conveyed, that is to say, as inert matter for the time being. The lodgment in the lymph nodes is probably due to mechanical agencies, the nodes acting as filters and barriers.

Here the bacilli begin to multiply and to set in motion that complex series of events leading to tubercle formation. Taking a tubercular focus in one of the thoracic lymph nodes of cattle, the first visible sign of the presence of the bacillus is the proliferation of epithelioid cells, with single nucleus or multiple nuclei. This new tissue undergoes central necrosis and caseation. The surrounding tissue proliferates to form a more or less dense capsule and the process comes to a standstill.

If we endeavor, with the aid and guidance of existing knowledge, to construct a sequence of the factors which are concerned in this process of tubercle formation, we shall find it extremely puzzling. It has occupied my attention for a number of years, yet even to-day with the help of the many currents of experimental data coming from so many laboratories I realize that we may choose several widely different interpretations without coming into violent collision with what we may regard as reliable experimental data.

At the outset it may be said that the tissue proliferation in tuberculosis is something specific in character, varying slightly

from host to host. In man and cattle it is much the same. In the smaller animals, either spontaneously diseased or inoculated, the tuberculous tissue is still characteristic, but giant cells are rare or absent. These, as a rule, are absent when the process is very rapid. In those species to which the bacillus has adapted itself, man and cattle, the cell proliferation is most uniform and characteristic.

The tissue reaction leading to the quiescent focus above described I believe to be a mechanism of defense for the body, even though imperfect. I also believe that it is a mechanism of defense for the tubercle bacillus—a mutual product, as it were. The structure of the tubercle interferes with the further dissemination of the bacillus by clogging the channels of escape. The bacilli become embedded in the proliferating cells and the necrosis protects the surviving ones from further attack for the time being. We can conceive that if this cell proliferation is somewhat delayed, the final result is a much larger focus. If it is still more delayed, the bacilli may be carried into other lymph nodes of the series and may establish several foci. The stimulus for such proliferation rests somewhere with the bacillus. It was shown many years ago by Prudden and Hodenpyl, by Straus and by many others later that dead, even boiled and washed, bacilli stimulate cell proliferation of a more or less specific type. We also know that such proliferation goes on in the presence of living bacilli, for an indefinite number survive the whole process.

In view of the fact that living, though very attenuated, tubercle bacilli are far more effective in producing immunity than dead bacilli, a fact brought out by Behring, Koch, Trudeau and others, we are safe in granting that the formation of the tubercle is stimulated by something given off from the living bacilli and not destroyed by heat. The simple stimulation of cell growth by the multiplying bacilli, however, does not fully explain the matter. There is an additional element which enters here, and this probably resides in the blood and to a less degree in the lymph. The blood is evidently an unfavorable medium, as indicated by the location of tubercles in the

various animal species.⁷ In order to account for the facts as nearly as possible, the following theory has been evolved during the past seven or eight years:

The tubercle bacilli as they come directly from some discharging focus are provided with some protecting, more or less inert substance as an envelope. This envelope maintains a neutral chemotaxis until the bacillus reaches the connective and lymphatic tissue, where it settles down. The protecting envelope is slowly removed by the normal tissue fluids. When this has been accomplished the bacilli are able to multiply, but during multiplication they stimulate cell proliferation and, according to the activity of this process, the multiplication is checked. The bacilli are destroyed in part; the rest, through the protecting influence of caseation, remain latent, provide themselves with the protecting envelope, and if discharged outward are ready to infect another individual.

It will be noticed at once that the theory presented has much in common with the theory of opsonins which A. E. Wright has developed with so much skill and industry since 1902.⁸ We may as well call the blood factor the opsonin, in deference to Wright, as the one who first called attention to it as a normal element. I do not agree with Wright, however, in attributing any special rôle to the blood leucocytes in body defense, for there is little or no evidence of this in the tissue reaction to the tubercle bacillus.

The many observations which refer to an early phagocytosis by leucocytes when bacilli are injected may be explained in two ways: First, as due to neglected factors inherent in the cultures used. These are the injection of too large numbers of bacilli, attenuated by long cultivation, and many of them dead, autolytic products, and the production of trauma during inoculation. The attenuated and dead bacilli fall a prey to

⁷ Maragliano states that he has been able to cultivate tubercle bacilli successfully only on guinea-pigs' serum.

⁸ Bulloch: Practitioner, Nov. 1905; a general summary and bibliography of Wright's work.

the leucocytes, and as soon as these are disposed of the true tubercle appears. Second, it is possible that the virulent tubercle bacilli may be carried by leucocytes, as are inert particles of dust, pigment and mineral, and deposited in a favorable place. We should scarcely attribute much importance to the carrying of a particle of quartz dust from the alveolus of a cow's lung to a mediastinal gland as a protective measure.

Baumgarten justly calls attention to the errors lurking in the injection of large numbers of bacilli, and his theory that the elements of the tubercle are quite different from the wandering phagocytes has been fully sustained. The phagocytosis which appears to go on in the tubercle itself I regard as a hedging in, a suppression of multiplication rather than a destruction. Even the common appearance of bacilli in epithelioid and giant cells may be interpreted as a growing around the bacilli on the part of the proliferating elements rather than an actual ingestion. That destruction may finally occur is highly probable, but necrosis soon ensues to check this and protect the remaining bacilli. I am aware that this assumption of a protecting envelope which can be conceived of as a secretion may appear strained, but I have been unable to harmonize the facts with any other theory. In its support I presented in a recent paper facts observed in the cultivation of tubercle bacilli, some of which I quote here: *

In the cultivation of tubercle bacilli the peculiar behavior of the bacilli first and last is best explained by assuming some change in the envelope or outer membrane of the bacilli. It is well known that it is very difficult and frequently impossible to obtain cultures of tubercle bacilli from tuberculous tissue in culture media in which they will grow readily after months or years of artificial cultivation. To obtain original cultures it is necessary to approximate as closely as possible the conditions obtaining in the animal body.

We can interpret this great change which the bacilli undergo in artificial cultures in two ways: 1. They make use of substances which at first could not be utilized as food. In other words, their metabolic

* Trans. of the First Annual Meeting of the National Association for the Study and Prevention of Tuberculosis. 1905.

functions have undergone a profound alteration. 2. The bacilli under artificial cultivation have eliminated something which has interfered with active absorption and assimilation.

I am inclined to accept the second theory and to assume that in the course of artificial cultivation a relatively impervious protective capsule has been gradually eliminated or modified, and, as a result, the growth and multiplication have become freer and more rapid. This elimination or modification of the envelope may go on by a selective growth of those bacilli which are most easily affected, or else the membrane may become modified in all bacilli because the active struggle with living tissue is in abeyance.

This theory also voices a condition which, I think, should be considered in any theory of immunity. I refer to the latency of tubercle and other bacteria in tissues. This latency of tubercle bacilli in lymph nodes has been demonstrated by not a few experimenters (Loomis, Pizzini, Harbitz and others). According to the theory here proposed, the tubercle bacilli are unable to multiply in the system when the opsonic power is too low, for the reason that the protecting capsules are not removed. Under such conditions the body is apparently immune, but really is in a state of hypersusceptibility. When the opsonic power rises the multiplication begins. This theory would also explain more rationally the greater activity of tuberculosis in certain decades of life.⁹

It is far from my purpose to apply this theory to all invasive bacteria. Each group or species possesses certain morphologic and physiologic peculiarities which are overdeveloped or suppressed in the evolution of parasitism. The work of Denys, of A. E. Wright and of Neufeld has shown that, while lytic forces may control typhoid and cholera bacteria, they do not govern streptococcus and other infections, in which cellular activity in the form of phagocytosis plays an important part. The more rapidly growing bacteria may possess quite a different mechanism of defense. As pointed out by Dr. W. H. Welch, they may secrete substances in the body which we do not sense in the

⁹ This conception of a hypersusceptibility in the form given here is, I think, new. There are several other diseases in which this conception may prove explanatory and stimulating, and I hope to return to this subject in a later paper.

culture tube. These he calls toxins, while I should prefer to call them protective substances of the bacteria. According to either conception, they would be harmful, the toxin directly, the protective substance indirectly by neutralizing the protective substances of the animal body. Finally, the theory here presented holds only for the spontaneous disease of man and cattle, attacked by their own specifically adapted races of bacilli. In experimental work this mutual relationship is disturbed by the foreign character of the bacilli used, by the crude methods of causing infection and by the use of artificial cultures more or less modified.

III. THE PRODUCTION OF SPECIFIC ARTIFICIAL IMMUNITY TOWARD TUBERCLE BACILLI.

The overshadowing problem before society to-day is that relating to acquired immunity to tuberculosis in the individual and its influence on future generations. Can immunity be induced artificially and will the survivors transmit anything of value to their offspring? The relative mildness of endemic diseases has been at times referred to as indicating the inheritance of acquired immunity, but the increased resistance of the population to endemic diseases can be explained as a result of weeding out or selection. In a recent lecture I pointed out that, with the weeding out of the host, there goes on a weeding out of the parasite as well until two are eventually selected which maintain a kind of equilibrium toward each other. During the weeding out of the host the parasite must gain in power to keep up with the former. This selected race of bacteria or other parasites attacking a population hitherto unexposed to it may cause serious epidemics and lead to the belief that the permanently infected population had gradually inherited an acquired immunity, whereas selection may have done it. In spite of these discouraging possibilities, the face of medicine and of society in general is determinedly set toward the prevention and cure of consumption and every possible means will be tried to raise the resistance of the individual. To experimental medicine has fallen the task of seeing what

can be done to raise the specific immunity artificially by making use of the tubercle bacillus, or any of its component substances, or even of hypothetical antibodies.

Historically the most important factor in the study of immunity is Koch's old tuberculin. From this, logically and illogically, all other methods of inducing immunity have radiated. It will be necessary, therefore, to deal with this first of all. The administration of this substance demonstrated three remarkable phenomena: 1. The great sensitiveness of the tuberculous individual and the comparative indifference of the healthy body to it. 2. A distinct thermal reaction of the tuberculous individual, that is to say, a general effect, and 3, a hyperemia of the tuberculous focus. These can be readily demonstrated on tuberculous guinea-pigs.

My interest in the tuberculin reaction was aroused in 1898, when I was giving considerable attention to the immunizing effect of tubercle bacilli, killed at the low temperature of 60 degrees centigrade. I was very much surprised to find that some of the guinea-pigs which had been inoculated with heated bacilli and in which there were no signs of active tuberculosis after eight weeks succumbed promptly to small doses of tuberculin. Others lost considerable weight, but survived.

This tuberculin reaction is closely allied to another brought out by dead bacilli, injected into an animal which has already received a dose of dead bacilli. In other words, the first dose of dead bacilli sensitizes the animal to such a degree that the second arouses a violent reaction and may even prove fatal. This had already been pointed out by Straus. Since making these experiments I have asked myself the question again and again, Why does a single dose of the dead bacilli sensitize the animal, and why does not a corresponding dose of tuberculin do likewise? So far as I was able to examine the literature, no one had succeeded in making animals hypersensitive by the use of tuberculin alone.

This hypersensitiveness I looked on as an immune reaction. The animal had been taken out of a condition of neutrality or indifference into one of irritability and defense, however imper-

fect. In seeking an explanation of this peculiar difference between heated bacilli and tuberculin, it became necessary to determine what the tuberculin reaction means. It is well known to the special student that there are about nine or ten theories of the action of tuberculin in print, and it seems perhaps folly to add another. Fortunately the one I believe accounts best for the facts observed by others and myself is very much like one of these nine or ten as I shall point out.

In the tubercular tissue and its immediate vicinity the tubercle bacilli have induced certain tissue changes, and with them certain new functions of the tissue have been aroused, which are the result of immunization. These new properties are concentrated in the immediate neighborhood of the focus. The specific resistance is, as it were, chiefly focal and only secondarily generalized. When the tuberculin comes in contact with this focus, the former is acted on, with the result that the originally innocuous tuberculin becomes poisonous perhaps by the splitting off of some poisonous substance. An incomplete digestion I should prefer to call it. As a result of this action we have, first, the local hyperemia and, second, the constitutional effect. In other words, the tuberculin becomes poisonous by an immune reaction directed toward the tubercle bacillus. This reaction is defective and in so far dangerous to the host. The only way in which the danger can be met is for the body to produce an antibody to this second substance. So far there is little evidence to show that the body is able to produce this in any amount. The animal body has learned to protect itself by suppressing multiplication rather than by attempting to neutralize such poisons.¹⁰

This theory of the tuberculin reaction as stated above is similar to the one proposed by A. Eber¹¹ nearly ten years ago. According to him the action of the tubercle bacillus raises the physiologic activity of the tissue involved in disease to such a

¹⁰ This secondary poison is probably of the same nature as the aggressins recently brought forward by Bail.

¹¹ Deutsch. Ztschr. f. Tiermed, XXI, p. 34.

height that it becomes capable of acting on the tuberculin and splitting off from it a toxic pyrogenic substance called by him tuberculopyrin.

My own interest in the tuberculin reaction was aroused by the query why a dose of dead tubercle bacilli can make the body sensitive while a corresponding amount of tuberculin does not. The reason why the injection of tuberculin as such does not lead to a subsequent tuberculin reaction as a result of one or several doses lies in the fact that tuberculin after injection is distributed throughout the body. Each cell receives but a brief exposure to a very minute quantity and probably much is eliminated unused. When dead bacilli (or even living ones) are introduced they soon settle down, and, the process of disintegration being very gradual, the tissues in which they are deposited receive a continuous, even though infinitesimal amount of tuberculin from the bacilli, and as a result of this persistent stimulus over a small area the tissue becomes focally active.

If this theory be true, the effect of the old tuberculin in establishing resistance appears in a new light. It would, first of all, exercise its chief function of being converted into a poison. During this conversion it uses up the antibody of the tuberculous focus. The benefit to be derived from it would be, first, in stimulating the reproduction of this antibody in the focus and around it, and, secondly, to accustom the body gradually to the action of the secondary poison set free from it. According to Koch, its action is antitoxic, but in a very round-about way. Nevertheless, the use of the very minute doses now generally advocated may accomplish a kind of one-sided resistance, which large doses, as given in the past, might strain and even injure. This view would also oppose doses of tuberculin which set free enough toxin to cause fever, and this mode of administration has been interdicted.

This theory, furthermore, harmonizes with certain recent experiments which show that tuberculin reactions are diminished in severity when accessible foci are removed before injecting the tuberculin.

The gradual loss of the tuberculin reaction may be accounted for in one case by the tissues becoming gradually accustomed to the secondary tuberculin poison, in another by the subsidence of the active process and the gradual loss of the antibody production on the part of the healed or quiescent focus, in a third by a temporary exhaustion of the antibody which activates the tuberculin in the focus. It is well known that in cattle the reaction from a tuberculin injection following another similar one at a short interval appears earlier and is weaker and shorter in duration than the first or it may not appear at all. If twenty times the initial dose be injected reaction does occur, but here we may have other substances enter which were present in the small dose in insignificant amount. Just as in diphtheria toxin the toxon elements began to appear when we gave up the ten-minim fatal dose for the fifty or one one hundred in testing the strength of antitoxins.

That I should have given preference to heated tubercle bacilli as immunizing agents rather than to the old tuberculin is obvious from the theory of action of the latter which I have formulated. Fully a year before I began my experiments with tuberculin, Koch, in 1897, had issued his new tuberculin T.R., which consisted of the ground, unheated bodies of tubercle bacilli. This was a distinct theoretical advance on the old tuberculin and was abreast of the new views of immunity. The old conception of the direct curative action of tuberculin had been abandoned. The issuing of the bacilli *in toto* tacitly acknowledged that the body must become immune to the entire bacillus and its metabolic products, for as long as we do not know which substance of the bacillus plays the most important and decisive rôle in arousing the defensive reaction of the body we must inject all of them.

There have been many other investigators working along similar lines. Some have kept us regularly informed of their forward and backward movements in this puzzling territory; others have kept to themselves their wanderings. The literature has grown to stupendous proportions, and any one who enters this field with any suggestions or theories is certain to

do injustice to some precursor, for almost every possible interpretation has been stated somewhere before.

Among the more thorough and distinguished investigators a few may be mentioned. Maragliano has essayed immunity with the watery extract of tubercle bacilli and has studied assiduously the various toxins of the bacillus. Denys has tried to immunize with the bacteria-free filtrate of cultures. There have been notable contributions to the chemistry of the tubercle bacillus by Kühne, Ruppel, Levene, de Schweinitz and others. The noteworthy work of Trudeau, Baldwin and their associates has greatly contributed to the steadying of our advance in the knowledge of immunity and its bearing on clinical medicine. These observers have also been untiring in separating the wheat from the chaff of that which has come to us from abroad. Very recently Behring announced the use of tubercle bacilli for immunization or treatment which, according to brief reports, have been extracted with water, 10 per cent. salt solution and, finally, alcohol, ether and chloroform. With this bacillar skeleton, as it were, he expects to obtain better results. The details of the process are not yet generally known.

The attempts at the preparation of a therapeutic serum I shall pass over, since there does not yet appear to be a very satisfactory experimental basis for estimating its efficiency. It will in any case remain of merely theoretical interest in the cure of tuberculosis, owing to the difficulty of preparation and the probable cost.

In spite of this array of painstaking contributions to the biochemistry of the tubercle bacillus and the relation of its various component elements, secretions and metabolic products to the production of immunity, we still appear to be at the beginning. The recent studies of Koch, Behring and Pearson in bovine immunity produced by the intravenous injection of living human bacilli, and the same experiments of Trudeau on smaller animals, bring us back to the old principle first brought out by Pasteur in 1880 in his studies of protective inoculation toward fowl cholera. We have not only retraced our steps to the whole bacillus, but even to the living attenuated bacillus.

A very pertinent question, one which has undoubtedly been put by every physician and experimenter dealing with tuberculosis, suggests itself here: If immunity does not appear in the course of tuberculosis, why should we expect to produce it by artificial means? An answer to this question involves many factors, on only one of which I shall touch.

Immunity in tuberculosis consists of two elements, the focal or local immunity due to the multiplication of tubercle bacilli in a given territory, and a less pronounced general immunity due to the biochemical activities of the local process. If the general immunity becomes quite strong, or if the original resistance is so great that a little impulse makes it complete, then a second attack is not likely to occur. This, alas, is not ordinarily the case.

Granted that the first infection manifests itself, as a rule, in certain lymph nodes, two different results may be looked for. Either it leaves an immunity which promptly fixes the next invader, closes in on him so that multiplication is speedily checked, or else in the less responsive the second invaders, lodging in the lungs themselves, may prove disastrous, owing to the destructibility of the lung tissue itself and the chance for secondary infections. This would mean that in the first type of individual the early infection protects against a second; in the second type, the first apparently, but not really, predisposes toward a second, the distinction being due to a difference in the rousing of immunizing factors.

In cattle the short life of the individual does not enable us to realize much from a study of primary and subsequent infections, but the impression that I have gained from a careful repeated study of the autopsy notes is that old lymph-node tuberculosis is rarely associated with fresh pulmonary disease. Cattle, I believe, are nearly immune and it requires but a little to tip the scales in favor of the host.

The acquired general immunity following the first attack is shown in a variety of ways. Experimentally the second local lesion in the guinea-pig, as pointed out by Koch, is a different process from the first. Clinically, the lymph-node tuberculosis

of childhood later becomes an organ tuberculosis. The bacilli are literally held up in the portal of entry, and pulmonary disease becomes the type of the second stage or of later life. The first infection of the intestines lodges and multiplies in the mesenteric lymph nodes. When lung disease is established and the sputum is swallowed, tuberculous lesions of the mucous membrane are very common; those of the lymph nodes, slight or absent. Behring is quoted by some one as stating that this infection is due to a hypersensitiveness. I should say a partial immunity, for here also the bacilli are held up at the place of entry. These facts were noted long ago, but not explained, by Weigert, as stated at the beginning of the article.

To the physician this phenomenon of repeated infection meant no immunity. And, indeed, so far as the patient is concerned, it is as good as none. It is more dangerous owing to secondary infection, but it carries in it the germ of possibilities, namely, the immunization to a degree which will totally prevent the second attack. In the meantime it may not be amiss to point out here the true significance of protecting patients from repeated infection. I should place this among the most important of the details of treatment, and it is not to be denied that the careful protection afforded tuberculous patients nowadays in sanatoria may have a powerful influence in raising the percentage of recoveries. To this opportunity for repeated infection on which I would place much more responsibility than on diffusion of early or latent infection in the body itself, there must be added the chance of acquiring tubercle bacilli of much higher invasive powers and, therefore, more dangerous.

At this point I may be permitted to digress a moment to refer to the peculiar localization of tuberculosis in the upper or cephalic lobes of the lungs. Numerous attempts at explanation have been made chiefly on anatomic bases. Some would make pulmonary disease hematogenous in origin, the infection coming from some disrupted primary focus, probably a caseous lymph node. The following evidence offered by experimental and comparative pathology on this puzzling phenomenon is somewhat contradictory, but suggestive.

I have already stated that in the spontaneous disease of cattle the largest or caudal lobes are most frequently diseased primarily or else those lymph nodes associated with them. The smaller cephalic lobes are more often secondarily diseased from aspirated caseous matter. This is as we should expect to find it if the germ lodges and multiplies where we should expect most bacilli to lodge when carried in the air.

Some years ago I noticed in several rabbits which had been inoculated into the ear vein with human tubercle bacilli and kept a long time the following peculiar condition: The bacilli which ordinarily are deposited in every part of the lungs and which, if virulent, fill the entire lung tissue with tubercles, had been suppressed and destroyed excepting along the thin border of both cephalic lobes, which were solid and tuberculous. It is probable that this condition can be frequently induced if the tubercle bacilli are very finely ground before injection so that large masses may not lodge and inevitably produce foci anywhere. These two facts, the spontaneous disease in cattle and the induced disease in rabbits, both favor, one negatively, the other positively, the hematogenic origin of tuberculosis of the upper lobes in man; but there is a third element in the form of a general principle which, to my mind, holds the balance. This may be briefly stated as follows:

Bacteria multiplying by preference in certain localities as *loci minoris resistentiæ* will reach these places if they have access to them through the blood or through natural openings. For example, typhoid bacilli injected into the blood will probably cause ulceration of the intestinal lymph apparatus just the same as if ingested. Applying these various data to pulmonary disease, there is no more reason to assume the suppression of tubercle bacilli entering by one route, the air, than by the other, the blood. I am myself inclined to believe that the bacilli inhaled are suppressed and destroyed except in the apices in susceptible individuals.

To return to our subject of focal immunity. This, as contrasted with a general resistance, is probably the chief stumbling block to successful artificial immunization. To bring

about the latter the whole body has to be exposed to the immunizing (and toxic) substances, as there is no other way of reaching certain avenues or portals of entry which are exposed to invasion. We might, for instance, cause the inhalation of an impalpable dust or spray of ground tubercle bacilli to increase the resistance of the lungs in the healthy and the diseased, but then the greatest care would have to be exercised not to give an overdose to an affected lung; otherwise a very severe or even fatal congestion due to the local tuberculin reaction might result.

This problem of local immunity and its relation to a general immunity has occupied my attention for a number of years. Beginning in 1898 I carried out a long series of experiments on guinea-pigs with bacilli killed at 60 degrees and 100 degrees centigrade to see how far a focal or local immunity contributed to a general resistance. These experiments have been frequently interrupted and are still incomplete partly because the equipment needed to protect attendants has not been available. So far only dead bacilli have been employed throughout. The animals used were guinea-pigs. No striking results have been obtained, and hence the work has remained unpublished. The experiments bear, however, on the subject before us and I shall briefly refer to them here.

If we inject a certain amount of a suspension of tubercle bacilli in some indifferent fluid, and killed at 60 degrees centigrade, into the peritoneal cavity of guinea-pigs, no immediate effect is produced. There may be at first slight loss in weight or none at all. After four to eight weeks the guinea-pig, outwardly well, is sensitive to tuberculin. An ordinary dose may kill it or reduce its weight considerably. At this time the peritoneal cavity may or may not show any local proliferation. In the omentum some nodules may be found centrally disintegrated, soft, like pus, but consisting only of the usual fatty débris. The inner wall of the nodule is smooth, there are no signs of a progressive disease anywhere recognizable with naked eye or in sections with the microscope.

If we now inject a second similar dose, the guinea-pig within

twenty-four hours begins a prolonged tuberculin reaction associated with fever and rapid loss in weight. If we examine the peritoneal cavity after one or more weeks, we now find considerable hyperplasia of the omentum, more rarely eruptions on the peritoneum of the abdominal wall. The omentum may become very large and adhesions may bind it to various organs, especially to the upper small intestine and lead in some cases to intestinal hemorrhage and rapid diminution in weight, even death.¹²

This second severe reaction I regarded as due to the rousing of a local immunity by the first reaction. The bacilli first injected into a neutral territory were probably largely carried off into different tissues before any local reaction took place, for I found histologic traces of their presence in the liver, spleen and bronchial glands. The phenomenon is thus similar to the invasion of the lymph nodes in the neutral body. At the same time many bacilli remained in the omental tissue and in the immediate neighborhood of the abdominal cavity. The second injection caused a prompt reaction on the part of the tissues first invaded and the bacilli were largely held there; hence the great proliferation of the omentum.

This increased local reaction following invasion is a general phenomenon, not limited to tuberculosis. When animals of more than the usual resistance are inoculated with septicemic organisms, the local reaction is always more severe and the disease more prolonged than in the most susceptible. If very susceptible animals are first partly immunized, the local reaction following the test inoculation grows more severe, parallel with the immunity, up to a certain point.

The question now arose, How much immunity have other distant tissues gained by this intraperitoneal local vaccination? If more or less general resistance is gained, why not induce

¹² Since making these observations I have asked myself whether some of the tubercular eruptions of the peritoneum, cured by operation, may not have been due to dead or nearly dead bacilli discharged from an old focus on a promptly reacting, because partly immunized, membrane.

with dead bacilli local foci in the periphery of the body under the skin, for example, where they can be controlled and watched? To test this, the abdominal cavity and the subcutis were used. A long series of guinea-pigs were inoculated, some into the abdomen, some under the skin, some with bacilli killed at 60 degrees centigrade, others with those killed at 100 degrees centigrade. The cultures were all relatively young cultures, both human and bovine, grown on dog's serum. The conditions were made as uniform as possible. In order to estimate the relative reaction caused by the subcutaneous and the intra-peritoneal injections the subcutaneous focus and the omentum were examined histologically. In general it may be stated that there was evidence that one injection had some influence on distant parts of the body, but this was distinctly below the influence imparted locally.¹³

¹³ In connection with these I tried to see if the injection of tubercle bacilli heated to one hundred degrees centigrade would produce any impression on a rapidly fatal infection with living bacilli. Eighteen guinea-pigs were used. In the first half of the experiment some received one injection of boiled bacilli into the abdomen, others, the same into the subcutis. After eight weeks they and controls received a surely fatal dose of living bovine bacilli into the abdomen. I knew that none would resist this dose and I simply wished to see what differences might appear. The abdominally immunized pigs lived longest, next came the subcutaneous cases and then the controls. The gain in prolonged life averaged only about seven days for the protected pigs. But the significant features of the experiment were exhibited in the course of the disease. The controls became feverish on the ninth day, yet even sixteen days later than this the vaccinated pigs were still well and active. Soon, however, they became ill and died suddenly. Here the immunity held the disease in check for a time, but when the resistance was finally overcome the process was very rapid. In the second half of the experiment, the guinea-pigs received two preliminary doses of boiled bacilli, one into the abdomen, the other under the skin. The same early checking of the disease was seen, a decided difference between treated and untreated being noticeable. All, however, succumbed; the average survival of the treated was about fifteen days longer than that of the untreated. The contrast would probably have been more striking if I had limited the test to a small number of fatal doses of bovine bacilli, rather than to the large dose actually given. Experiments similar to this have probably been made by many others before.

IV. SOME SUGGESTIONS CONCERNING THE PRACTICAL APPLICATION
OF METHODS TO PRODUCE IMMUNITY.

The experiments made on cattle with human tubercle bacilli by Behring, Pearson, Koch and others have shown that a pronounced resistance to living bovine cultures may be established even in young animals. This procedure is, of course, inapplicable to man. In the immunization of cattle two factors operate very strongly toward the success of the process: First, the intravenous injection of the bacilli which carries them to every part of the body and especially to the lung tissue where we know the bacilli are likely to be held back in large numbers. Here they are most needed to produce a local resistance in the most frequently exposed and diseased organs (lungs and lymph nodes) of the body. It is doubtful that the bacilli multiply at all after injection.¹⁴ In the second place, the postmortem examination of spontaneously and artificially infected cattle has led me to believe that cattle are in a fair state of equilibrium with their bacillus and that there is needed but a relatively slight impulse at the right place to establish a resistance which will promptly suppress the invaders.

The investigations of Nägeli, Necker and others which reveal a very high percentage of latent or arrested infection in the human subject also indicate that the normal human being possesses considerable resistance and that after infection only a slight impulse efficiently applied may suppress the disease at an early stage. This encouraging possibility leads me to believe

¹⁴ It is of interest here to note that according to Behring the intravenous injection of these human bacilli into adults may cause a fatal pulmonary edema, which we may explain as a local tuberculin reaction in infected animals. I described a similar condition in rabbits several years ago. After intravenous injection of certain cultures mid-way in virulence between the bovine and the human types, a fatal pulmonary edema and hyperemia may appear after four or five weeks. This is probably a true tuberculin reaction of the lungs, due to the extensive destruction of tubercle bacilli and the liberation of poisons. Similar pulmonary accidents in goats were described by Arloing.

that there is a great opportunity for some form of preventive inoculation before the disease has fastened itself on the predisposed subject, if such a process could be introduced. This vaccination should be equally applicable before and after infection. The general outcome of the investigation with bacilli killed at various temperatures has encouraged me to suggest their use for immunization. The very fact that they are so much more efficacious than the old tuberculin in rousing the antagonism of the body is significant. But it may be said the injection of dead bacilli will lead to a local focus, an objection which Koch tried to overcome at the start by extracting bacilli and so producing the old tuberculin. It may also be asked what advantage can there be over the ground and crushed bacilli which have been subjected to no heat whatever and which are now being used by A. E. Wright and others in the treatment of chronic skin affections. It may even be urged that immunity or increased resistance has been attained in exceptional cases by the repeated injection of the old tuberculin. Macfadyan and C. Sternberg refer each to such a case. A relatively high degree of resistance has been reported by Koch to be attainable with his new tuberculin TR. There can be no doubt that all the preparations emanating from tubercle bacilli or the culture fluids contain substances which induce some resistance. If the reaction of the body is made up of several factors, as I have endeavored to explain, then the strengthening of any one factor may favor the final resistance produced.

The advantages which, I believe, will flow from the use of bacilli killed at a low temperature, are twofold:

1. The creation of a local focus of ever so slight a character, let us say in the subcutis, may lead to the production of immune bodies which, radiating from the focus, may prove efficacious. These foci may be multiplied by simply changing the point of injection. It may be that the very objection urged by Koch against a local focus, namely, that the immunizing substances remain there, is the very essence of the whole process. At any rate, we have no reason for believing that the crushed or

ground bacilli or even the tuberculin TR. is diffused more rapidly after the reaction of the body has been roused. Levene found that even fats repeatedly injected subcutaneously finally led to a local reaction with induration. In looking over the literature recently I was surprised and gratified to learn that Maragliano had recommended some such mode of treatment in his Philadelphia address.¹⁵ Such local foci can be watched and their behavior correlated with the general subjective and objective symptoms.

The second advantage to which I wish to call attention concerns the material to be injected. In the production of immunity the tubercle bacilli to be used should be as recently isolated as possible and grown on blood serum to which pieces of sterile animal tissues may be added if desirable. If the theory I have advanced be true, that the body first acts on some product of secretion in the bacillus which has taken the form of a protective envelope, then the more recently isolated the culture and the more nearly the culture medium approximates the living body the more likely the active production of this envelope. This, of course, should be present in the bacilli to rouse to greater activity the antibody or opsonin after injection. The products in the market are prepared on a large scale from actively multiplying bacilli. A long experience leads me to the inference that there is an inverse relation between virulence and activity of multiplication. I have also pointed out that the slow accustoming of tubercle bacilli to media on which they at first absolutely refuse to multiply suggests the throwing off of some restraint (such as an envelope) either by all bacilli gradually or through the selection of a few which more quickly adapt themselves. The use of such early cultures of tubercle bacilli, grown on appropriate media, carefully killed at 60 degrees centigrade and tested with proper precautions before application, is within the reach of every hospital or sanatorium dealing with tuberculous patients.

My reason for presenting a method which I myself have not

¹⁵ Medical News, lxxxiv, 1904, p. 625.

tried is because I have no opportunities for such trial, and I am convinced that the delicate methods of immunization can not be successfully tested on any animals, except perhaps monkeys and cattle, and there are obvious objections and difficulties to be met in the use of either species. I believe that this method should at least be given a trial, although its execution will require considerable personal care and the observance of minute details which the medical profession is inclined to throw on the commercial bodies who manufacture biologic products, but from whom such kind of work can hardly be expected. Of especial importance is the test that the heated bacilli are actually dead, for the temperature of 60°C. is the critical temperature, below which tubercle bacilli are probably not destroyed. Experiments still incomplected indicate that bacilli killed in this way possess properties approaching the living organisms.

CONCLUSIONS.

In conclusion, I wish to allude briefly to the struggle against tuberculosis from the point of view of the bacillus itself, for the slight changes which this parasite undergoes are writ large on the history of mankind. By spying about the enemy's camp we may learn much for our own safety.

The tubercle bacillus is undoubtedly open to modification, and we may safely believe that there are a large number of races or varieties in existence. Even among the small numbers which we are able to study carefully in the laboratory, there are constant differences indicated by biologic and pathogenic tests. These must be the result of natural selection and brought together perhaps by the great immigration movements of the present era.

The thesis which I tried to discuss recently is that the tendency of infectious diseases is toward a balanced parasitism, with a reduced mortality, but not necessarily a reduced morbidity as a result. This is due to the selective adaptation of both host and microbe. For the latter the most important need is the establishment of a definite mode of entry and exit. In the case of the tubercle bacillus the chronic infection of the

lungs is the most favorable type of disease for the microbe itself. This selective adaptation will go much farther, I believe, and we shall undoubtedly meet with bacilli of very low invasive power which find a favorable nidus for multiplication in bronchial secretions. There is already some evidence that tubercle bacilli in sputum do not always signify serious consequences.

The only way to determine the relation between pathogenicity and character of the disease would be a study of the bacilli themselves. This would throw such an additional burden on clinical medicine that we can hardly hope for much progress in this direction. These very attenuated forms may become, as it were, the parasites of the sick lungs rather than of the normal ones. They would take the same position which pneumococci, streptococci and staphylococci occupy in the upper air passages.

The influence which a possible immunization of the human race might have on the destiny of the tubercle bacillus is open to debate. In the first place, all immunization is a confession that the parasite has broken through barriers and has come to stay. The only way to suppress an infection is to do so rather than to establish a compromise by simply increasing our resistance. The latter is admirable from every point of view, but it is not by itself going to eliminate tuberculosis. The only way to accomplish this is to prevent the bacillus from attacking a new subject. Immunization, combined with isolation and other preventive measures, would probably place a decided check on the disease, while immunization by itself alone would lead eventually toward the selection of especially virulent races of the tubercle bacillus which, producing a mild disease in the partially immune, would probably cause a very severe disease in the unprotected or unvaccinated. If the method of immunizing cattle now made generally possible by the commercial exploitation of Behring's bovovaccine should become widespread, we would be treated to a most valuable object-lesson of the effects of this process on the protected and unprotected. With the introduction of such a method there is likely to come a slackening of the usual preventive measures and a more indis-

criminate dissemination of tubercle bacilli followed eventually by the appearance of more virulent races.

In this fragmentary exposition of the parasitism of the tubercle bacillus I have left many phases of the subject untouched, many statements undeveloped and certain theories quite unprotected. My purpose in presenting them is not so much to produce conviction as to stimulate others either to develop them further or else to rout them and to put something better in their place. They may be regarded as working hypotheses through which I have attempted to correlate existing data, my own studies serving merely as a guide through the Babel of theories.

The best that the laboratory worker can do is to suggest principles or laws which must be intrusted to the clinician, if he will accept them, to be developed and applied to the many variations which actual spontaneous disease manifests. It is also true that the working out of methods in the laboratory and their clinical application are two wholly different problems. Experimental and clinical medicine must work hand in hand, with the closest co-operation, if one does not wish to disappear in pitfalls known only to the other.

THE CAUSE OF THE HEART BEAT*

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HISTORICAL REVIEW.

THE Seventeenth Century: Harvey and Willis.—It is known to all students of the history of medicine that what may be designated in general as the modern point of view regarding the cause of the heart beat dates from the work of William Harvey. Before his time physicians thought along the lines laid down by the ancient masters, in that they conceived the expansion of the heart in diastole to be an active, or rather the active phenomenon of the heart beat. This expansion was attributed to the innate or implanted heat, to the vital spirits, to the pulsatile force, etc. That the diastole of the heart is an active rather than a passive expansion has been held in a sense by many prominent physiologists, even in the nineteenth century. Magendie, for example, states that at first he entertained this belief. Nevertheless it remains true that Harvey's experiments and observations demonstrated that the pulse of the heart and the arteries is due to a contraction of the musculature of the heart during systole, whereby blood is driven forcibly into the arteries. "The motion of the heart consists in a certain universal tension, both contraction in the line of its fibers and constriction in every sense."¹ This conception of the heart as a muscular force pump, driving out its contents during its active contraction, instead of drawing blood and pneuma into itself by a spontaneous dilatation was, as Dalton says, the first important step in the development of the doctrine

* Lecture delivered March 17, 1906.

¹ Harvey: "The Motion of the Heart and Blood in Animals," Willis' translation, revised and edited by Alexander Bowie, London, 1889.

of the circulation. Certainly as regards the nature of the heart beat it makes the great dividing line between ancient and modern speculations.

We may regard Harvey also as the founder of the myogenic theory of the heart beat. For although Galen taught that the pulsations of the heart are dependent on an inherent force, which acts through the contractions of the peculiar tissue composing the heart, yet his conceptions of the nature of the heart beat were so different from ours that his ideas are scarcely applicable to the problem as it presents itself to us. Harvey interpreted the systole and the diastole as a contraction and relaxation respectively of the musculature, and, although he did not speculate as to the cause or nature of the contraction, he seems to have considered it an inherent property of the heart muscle itself. Later writers attempted a further analysis of the phenomenon, and in one form or another hypotheses arose, which attributed the initial cause of the heart beat to the activity of the nervous system. Willis² taught that the nerves supplying the heart, stomach, intestines, etc., arise from the cerebellum, and that, therefore, this organ engenders the animal spirits through which the involuntary movements of the heart are effected. This view found many advocates, but it disappeared in the course of time, after it was shown that the nerves in question do not originate in the medulla, and that so far as the heart is concerned, the beats continue even when the organ is excised. Borelli³ devised an ingenious hypothesis by means of which he reconciled the last mentioned fact with the view of a nervous control of the heart beat. He assumed that the contractions of the heart depend on an influence derived through the nerves. The liquid, *succus spirituosus*, conveyed by the nerves, escapes slowly into the heart and sets up there an ebullition, which is the immediate cause of the contraction, or according to his view, the expansion of systole. Since in the excised heart some of this liquid is still contained in the stumps

² Willis, Thomas: "Opera Omnia," Amsterdam, 1682. (Budge.)

³ Borelli: "De Motu Animalium," 1743.

of the adherent nerve fibers, it is evident that the pulsations of the organ may continue for a time after removal from the body.

The Eighteenth Century.—Evidently the century following Harvey's immortal discovery did not add anything of material importance to our understanding of the cause of the heart beat. But the tendency manifested during this period to attribute the initiation of this phenomenon to some direct influence of the nervous system was of value indirectly, in that it led to continual discussion and to some experimental work. Haller devoted much attention to the problem and he succeeded apparently in disproving the neurogenic views in the form in which they had been proposed at that time. In place of them he formulated a clear-cut myogenic hypothesis which served to mark the beginning of a new discussion lasting until the present day. He insisted on the fundamental facts that the heart continues to beat after section of the nerves going to it and even when "torn out of the breast." The musculature of the heart, he says, possesses an inherent irritability or power of contraction, which is independent of the influence of the extrinsic nerves; a view, which to his mind, was demonstrated by the fact that a quiescent heart may be excited to movement by "heat, vapor, cold, poison, current of air, watery liquids, wax, blood, or an electric spark."⁴ According to his theory, therefore, the cause of the heart beat lies in the inherent irritability of its musculature, and under normal conditions this property is aroused to action by the stimulating influence of the blood as it flows into the ventricles from the veins and auricles. "By this blood copious, warm and heavy, the sensible flesh of the heart is irritated and excited to contraction." For a time Haller's views met with general acceptance, but some of his contemporaries remained unconvinced. Senac,⁵ for example, while admitting that the heart is essentially independent of

⁴ Haller: "Elementa Physiologiæ Corporis Humani," vol. i, 1757; also First Lines of Physiology. (Cullen.)

⁵ Senac: "Traité de la Structure du Cœur," etc., 1774. (Budge.)

the central nervous system and is made active by the stimulating effect of the blood, conceived that this stimulus acts on the nerves in the heart, rather than on the musculature directly.

The Nineteenth Century.—About the commencement of the nineteenth century physiology began to assume definitely the characteristics of an experimental science. The greatest masters had, indeed, always made use of experiments in their inquiries, but their disciples, on the contrary, were more industrious in the matter of argument and exposition than in devising new investigations. The dawn of a new day for our science was slowly breaking at the close of the eighteenth century. Henceforward, experimental work became more widespread and physiologists adopted promptly all the available methods and appliances that were developing in the sister sciences of chemistry and physics. So far as our subject is concerned, one of the first evidences of the awakening of this experimental spirit is to be found in the numerous attempts made to determine whether or not the beat of the heart is influenced by the cardiac nerves. These nerves were stimulated or severed and the effects on the heart beat were observed. Senac, Bichat, Fontana, Treviranus and others reported negative results from the stimulation of the cardiac nerves, while von Humboldt, Burdach, and later Longet and Valentin, stated that an increase in the heart beat was obtained.

Many of us may wonder why these capable observers failed to discover the inhibitory action of the vagus nerve on the heart beat, that phenomenon which we now obtain with such ease and certainty that it constitutes one of our usual and always successful class demonstrations. The explanation is, I believe, very evident. These men possessed only crude and inefficient methods of stimulation, such as mechanical compression or section, the action of chemical agents, the electric spark of the Leyden jar or the statical machine, or the continuous current of the voltaic pile. If we were compelled to resort to the same appliances now it would be difficult or impossible for us to demonstrate with certainty such an effect as the inhibition of the heart by stimulation of the vagus nerve.

In this, as in many other respects, experimental investigations in physiology were immensely aided by the discovery of the induction current, and the construction of instruments capable of giving a rapid series of induction shocks. Magnetic induction currents were first obtained by Faraday, in 1832, and shortly afterward he succeeded in developing similar effects from the current of the galvanic battery, that is, in producing what he called voltaic induction. Both of these discoveries were quickly applied to the uses of physiology. With the aid of a magneto-electric rotator, the brothers Weber,⁶ in 1845 and Budge,⁷ perhaps independently, in 1846, succeeded in demonstrating the inhibitory action of the vagus nerve on the heart. One of the three talented Weber brothers, Wilhelm Weber, was a distinguished physicist, and it is stated that in 1838 he devised a form of magnetic rotator which was capable of giving a series of induction shocks. Possibly it was through his influence that his brothers, Ernst Heinrich and Eduard Friedrich, were led to use this apparatus in their investigations on the vagus nerve.⁸ This fundamental discovery not only opened a new field to physiological thought and investigation, but it also disposed finally of the erroneous view that the vagus nerve serves as a motor channel through which the central nervous system controls and originates the beat of the heart.

The general view that the beat of the heart is directly dependent on the central nervous system had meanwhile taken another form. On the basis of excellent experimental work Legallois⁹ concluded that destruction of the spinal cord is followed by a failure in the power of the heart to maintain its contractions; and, indeed, in animals not too young, this result was obtained when any given region of the cord, cervical, dorsal or lumbar,

⁶ Weber: Wagner's Handwörterbuch d. Physiol., vol. iii, No. 2, p. 31. See also Volkmann, *ibid.*

⁷ Budge: Müller's Archiv., 1846, p. 295.

⁸ According to Schiff, the credit of first observing that stimulation of the vagus nerve arrests the heart-beat belongs to Galvani. I have not been able to verify the reference.

⁹ Legallois: "Experiences sur le principe de la vie," 1812.

was alone destroyed. We know now that such operations may be performed, with the aid of our better technique, without causing the death of the animal, and it is probable that the fatal results obtained by Legallois were due directly to vasomotor paralysis. He was convinced that his experiments proved that the stimulus or principle which maintains the beat of the heart is derived from the spinal cord in all of its parts, and is conveyed to the heart through the branches of the sympathetic system.

Legallois's experiments and conclusions were passed on and endorsed by a special commission, consisting of von Humboldt, Hallé and Percy, appointed by the French Academy; but the dictum of a commission, fortunately, is not accepted as final in science. The work of Philip and of Clift in England; of Flourens and Brachet, in France, and of Bidder, in Germany, soon made Legallois's conclusions doubtful, or altogether improbable.

Throughout this period, during which efforts were made to show that the heart beat is controlled directly from the central nervous system, most physiologists were saved from the intricacies and perplexities of obscure and doubtful interpretations by holding fast to the simple, incontestable fact that the heart continues to beat after removal from the body. This fact, says Volkmann,¹⁰ proves absolutely that the force causing the heart beat does not arise in the brain or cord. The development of modern physiology has served to demonstrate the correctness of Volkmann's reasoning. By the methods of isolating the heart first used in this country by Newell-Martin, and subsequently modified and improved by Porter, Langendorff, Locke, Hering and others, we are now able to keep the hearts of mammals, including man, beating for hours or days, after all connections with the central nervous system are severed.

That form of the neurogenic theory which made the heart's beat dependent on the central nervous system was finally

¹⁰ Volkmann: Müller's Archiv., 1844.

disposed of when the true functions of the extrinsic nerves to the heart were demonstrated. Physiologists were perhaps more willing to let it depart forever, because, at this period, an entirely different kind of neurogenic hypothesis was meeting with great favor on all sides. According to this newer view, the cause of the heart beat lies in the automatic activity of the sympathetic ganglia, present in the heart itself. In this form the neurogenic hypothesis quickly and completely replaced the myogenic theories as proposed by Haller, the pendulum of physiological belief taking a strong and a long swing to the nervous side. This particular form of the neurogenic theory seems to have originated in the work of Bichat.¹¹ In the grand and comprehensive system devised by this remarkable man, the processes of the animal body are divided into the animal and the organic. Over the latter, including the beat of the heart, the central nervous system has no control; they are under the influence of the sympathetic or ganglionic system. The sympathetic nerve in fact was assumed, in accordance with the old idea of Winslow, to consist of a number of small independent systems for each of which a ganglion served as a center or brain. No direct evidence was furnished then, nor has it been furnished since, that such ganglia control the movements of the heart, but the theory offered a plausible hypothesis, and, in the minds of most physiologists, the hypothesis was almost definitely proved when, in 1844, Remak¹² described nerve ganglia within the substance of the heart. From our modern standpoint it is difficult to understand why this anatomical fact should have carried so much weight.

If the isolated heart continues to beat it is surely just as logical to assume that the cause of the beat lies in the properties of the musculature, as in those of the intrinsic nerves. Yet for a long period of years physiologists were practically unanimous in giving the latter interpretation. The consideration that had most weight with them beyond doubt, was the orderly

¹¹ Bichat: "Récherches physiol. sur la vie et la mort," 1800.

¹² Remak: Müller's Archiv., 1844.

co-ordinated character of the heart beat. The co-ordination of different muscles to give an orderly sequence of contractions is usually under the control of the nervous system, as is shown in voluntary or reflex movements and in the rhythmic play of the respiratory muscles. In the heart beat a similar sequence is presented. The contractions of the veins, auricles and ventricles follow in a definite order, and whether stimulated naturally or artificially the musculature of each chamber contracts in a co-ordinated fashion. For the control of such a complex and yet orderly movement it was natural, reasoning from analogy, to assume that nerve centers are required. Practically all physiologists abandoned the myogenic hypothesis of Haller and accepted the neurogenic theory as formulated by Volkmann.¹³ For a period of 40 years this view was taught almost exclusively in the text-books and was accepted in all the discussions of the current literature. Investigation, in fact, was directed largely toward discovering the finer anatomy of the intrinsic nervous mechanism and speculating on the specific rôles played by the different ganglia.

In the frog's heart, which formed the classical object of research, two distinct nerve centers were described, one, the ganglion of Remak, situated at the junction of the sinus venosus and auricles, the other the ganglion of Bidder, lying at the level of the auriculoventricular ring. Numerous experiments were made to determine the difference in function, if any, between these two centers. While the original view had assumed simply that the intrinsic ganglia function as motor centers, discharging impulses in regular sequence into the different chambers, numerous corollaries or amendments to this hypothesis soon appeared. For a time the polydynamic theory, as it was designated, obtained increasing acceptance. It was recognized that the beat of the heart begins normally at the venous end and thence spreads in orderly sequence to auricles and ventricles; hence it was concluded that the ganglion of

¹³ Volkmann: *Ibid*; also Wagner's *Handwörterbuch d. Physiol.*, vol. ii.

Remak in the sinus constitutes the original or chief motor center in which the first impulses arise automatically. But it was known also that parts of the heart, separated from this ganglion by section or ligature, may also beat rhythmically and respond to artificial stimulation by co-ordinated contractions; hence it became necessary to regard other nerve cells in the heart as subordinate motor centers. Bidder¹⁴ and others believed that the ganglia discovered by him at the junction of auricle and ventricle function as reflex motor centers. So also the inhibitory action of the vagus nerve led naturally to the supposition that an inhibitory center exists in the heart; while numerous pharmacological studies suggested still more elaborate views in order to explain the differences in action of various drugs. Schmiedeberg¹⁵ (1870) assumed the existence not only of motor and inhibitory centers, but also of an intermediate apparatus of some sort placed on the course of the vagus fibers before they reach the inhibitory center. Perhaps the latest effort of this kind to differentiate between the functions of the intrinsic ganglia is found in the papers of Kaiser¹⁶ (1893-4) who describes an automatic or excitomotor center at the venous end of the heart, certain subordinate motor centers in auricles and ventricles and an inhibitory center in the auriculoventricular region. The last named center is stimulated reflexly by the systole of the ventricle, and then sends inhibitory impulses to the subordinate motor centers, whereby these latter are inhibited, and diastolic expansion is produced in the ventricle.

From the time of Volkmann to that of Kaiser the neurogenic hypothesis has been given many specific forms by Joh. Müller, Kürschner, Budge, Schiff, Goltz, and others, but in recent years the interest in this matter of subdividing the functions of the various ganglia has obviously subsided. It is recognized that the whole neurogenic theory is again under discussion, and

¹⁴ Bidder: Müller's Archiv., 1852.

¹⁵ Schmeideberg and Koppe: Berichte d. könig. sächs. Gesells. d. Wiss., 1870.

¹⁶ Kaiser: Zeits. f. Biol., 1893, p. 203; *ibid.*, 1894, p. 279.

that it is more important at present to decide the fundamental question, whether the nerve cells have anything to do at all with the cause of the heart beat. The neurogenic view held to-day by many physiologists probably asserts no more than was stated by Munk in 1881, namely, that the automatic ganglion in the sinus (in the frog's heart) is the *primum movens* of the heart's activity, and that the excitations proceeding from it through the septal nerves stimulate the auricle, and indirectly the ventricle through the activity of the non-automatic ventricular ganglia. The strict neurogenists must hold in addition that all the ganglion cells (or the elements of the nerve network) in the heart are capable of independent automatic or reflex activity, since isolated bits of the heart under proper conditions give rhythmic pulsations. Friedländer asserts that very minute fragments of the heart, not larger than 0.2 mm., when taken from the sinus, auricle or upper third of the ventricle, continue to beat as long as 48 hours, if kept in a proper serum.¹⁷ The proofs furnished for this neurogenic theory were not really conclusive, and it is, therefore, natural to find that after a certain period of triumph, some investigators began to scrutinize the facts in a skeptical way.

Engelmann, influenced by his experiments on the rhythmic contractions of the ureters, which are devoid of nervous elements, suggested in 1869 that possibly the heart beats have a muscular origin. But the modern renaissance of the myogenic theory dates from the work of Gaskell,¹⁸ from 1881 to 1883. He began his well-known experiments on the rhythmic activity of the heart in frogs and terrapins while still a believer in the neurogenic theory, but the facts that he discovered convinced him that the heart muscle itself possesses the property of automatic rhythmicity, and that this property is most highly developed in the tissue at the venous end of the heart. He proposed, therefore, a theory which, like that of Haller, assumes an

¹⁷ Friedländer: von Bezold's Untersuchungen a. d. physiol. Lab. in Würzburg, 1867.

¹⁸ Gaskell: Jour. of Physiol., 1883, vol. iv, p. 43.

independent irritability and rhythmicity in the heart muscle, but which differs from Haller's in that it does not attempt to account for the cause of the contractions.

Gaskell's beautiful experiments led him to believe that the systole of the heart begins at the venous end (sinus venosus), because the property of rhythmicity is most highly developed in this region. Thence the contraction spreads as a peristaltic wave over the rest of the heart, its rate of conduction being rapid in the expanded and modified portions of the original tube which constitute the auricles and ventricle, and less rapid in the more primitive tissue that forms the auriculoventricular ring. The slower velocity at this latter point occasions the apparent pause between the auricular and ventricular systoles. As for the nerve cells found so plentifully in the heart, he considers them merely as peripheral sympathetic cells in which the preganglionic fibers of the vagus end before being distributed to the cardiac muscle. They constitute, therefore, merely a portion of the inhibitory mechanism, and are in no way connected with the causation of the heart beat.

Gaskell's point of view was supported by Englemann in numerous excellent researches, and from that time the myogenic theory has won many adherents among the physiologists of all countries. The controversy still furnishes material to the current literature, and the fact that this difference of opinion exists at present is in itself evidence that no absolutely conclusive proof has been furnished by either side.

Some of the many arguments advanced by one party or the other have failed to stand the test of subsequent investigations, and have, therefore, dropped out of the case as it presents itself to us to-day, but the actual condition of the question may be understood most clearly by reviewing briefly those facts and deductions which are still used most frequently by the adherents of the two views.

FACTORS IN THE PROBLEM.

Automaticity of the Various Parts of the Cardiac Musculature.—Is it true that all parts of the heart possess the property

of giving automatic rhythmic contractions? We know that under normal conditions the beat of the heart begins in the great veins. The contractions of the remaining portions are due to impulses or excitations received from the venous end, either by way of the musculature or the nerves. Under normal conditions, therefore, only the venous end contracts automatically; the remainder of the musculature is stimulated to beat. When, therefore, we consider the automaticity of the heart beat as a whole, it is obvious that it depends for its initiation on the properties of that small portion of the musculature which forms the mouths of the great veins. The question arises whether the rest of the muscle of the heart possesses similar properties. Can any portion of it continue to beat rhythmically if its connections with the venous end are severed? The evidence that has accumulated within the last quarter of a century seems to me to be entirely conclusive on this point. It shows beyond question, so far as the vertebrate heart is concerned, that every portion possesses, in some degree or other, the property of giving automatic rhythmic beats provided the proper conditions are maintained. If the apex of the frog's heart is separated from the rest of the organ it refuses to beat notwithstanding that it is supplied with normal blood. But if it is placed under a certain tension, or if certain substances are added to the circulating blood, it will give rhythmic contractions.

When the terrapin's ventricle is cut off from the rest of the heart it remains quiet as a rule, so long as normal blood circulates through it, but a slight change in the inorganic salts of the circulating liquid, such as the removal of the potassium compounds, causes it to begin a series of beats. So, also, strips taken from various parts of the heart may be made to beat rhythmically for long periods by immersing them in special solutions. While it is true, therefore, that certain parts of the heart in some animals are incapable of beating automatically under perfectly normal conditions, that is, when supplied with the animal's own blood, it is evident that such portions of the heart have a latent property of automatic rhythmicity which

may be aroused into activity when the proper conditions are provided. The beats under such conditions are of the same order of phenomenon as those normally exhibited by the venous end of the heart. If, therefore, we could find any portion of the heart entirely devoid of nervous tissue and could so modify its conditions as to cause it to give beats of the usual cardiac type, we should be in possession of a fact that would be nearly conclusive proof of the myogenic origin of the heart beats. Very numerous efforts have been made to furnish a crucial experiment of this sort.

Rhythmic Contractions in the Absence of Nerves.—The following instances of rhythmic contractions in muscular tissues devoid of nerves have been cited. The ureters (Engelmann); the veins in the bat's wings (Wharton Jones); the veins in the rabbit's ear (Schiff); certain portions of the veins opening into the frog's heart (Engelmann); the apex and also the bulbus arteriosus (Engelmann) of the frog's heart; strips from the apical portion of the terrapin's ventricle (Gaskell); the hearts of many invertebrates (Foster, Biedermann, *et al.*); the heart of the young embryo (Wagner, His). Most of these instances carry but little weight, as proof of the myogenic theory, at the present time. In regard to the ureters, or the veins in the wing or ear, it may be urged that they prove nothing concerning the heart, even if the absence of nerve tissues were demonstrated beyond doubt. So also regarding the apex or bulbus arteriosus of the frog's heart, or the hearts of invertebrates, the results of histological work in recent years have tended in the direction of making untenable the long held belief that these tissues are entirely destitute of nerve cells.¹⁹

The old observation that the fetal heart begins to beat before it possesses nervous elements is the only one of the facts of this kind which remains unchallenged. Its correctness seems to have been clearly demonstrated in recent years by the careful

¹⁹ For discussion and literature see Carlson: Pflüger's Archiv., 1905, vol. cix, p. 51.

work of W. His, Jr.²⁰ Engelmann has cited this fact as the best single proof of the truth of the myogenic theory. Objections, however, have been raised to its conclusiveness. It has been urged that the fact that the beat of the embryonic heart is myogenic in origin is in itself no proof that the older heart, with its intrinsic nervous mechanism, continues to function in the same way. It may be that after the migration of the nerve cells into the heart they assume, as the more automatic tissue of the two, the function of initiating the beat. This mode of reasoning appeals to some physiologists. On the other hand it is pointed out that the negative evidence regarding the presence of nerve elements in the early embryonic heart may disappear, as has happened to such evidence in other cases, when better histological methods are devised. An improved technique may show the presence of nerve elements in the embryonic tissue, or demonstrate the existence of conducting paths between it and outlying nerve cells. The case at present gives strong support to the myogenic theory, but it has not sufficed to remove the objections of the other side.

Hearts Known to Be Controlled by Nerve Cells.—Among the great variety of hearts studied by physiologists some have been discovered in which the beat is obviously dependent on the presence and properties of nerve cells. These cases merit especial consideration. It is believed by some that the lymph hearts in the frog furnish an example of this kind. Destruction of the spinal cord is followed by a cessation of the beat, and light doses of curare have a similar effect; both facts indicating that the musculature is normally stimulated by impulses received from extrinsic nerve fibers. The case, however, is not a clear one, since according to some observers the hearts may begin to beat again after removal of the spinal cord, and moreover it is at most an instance of a heart controlled directly from the central nervous system and not by the action of peripheral

²⁰ His, W. Jr.: Abhandign. d. math. phys. Klasse d. könig. säch. Gesells. d. Wiss., 1891, vol. xviii; also Krehl and Romberg: Archiv. f. exp. Pathol. u. Pharm., 1892, vol. xxx, p. 71.

ganglia. A similar case is presented by the caudal hearts of the hag fish. Greene has shown²¹ that the beat of these peculiar structures is dependent on the action of a nerve center in the spinal cord. Destruction of this center brings the heart to a standstill.

The contractile apparatus in this case is not a true heart in structure; it is composed, rather, of two specialized striated muscles, belonging to the somatic type, which are so arranged as to compress and dilate two membranous sacs. As Greene says, the whole mechanism is strictly comparable to that of the respiratory muscles in mammals. It furnishes no more support to the neurogenic theory than is already to be found in the existence of the rhythmic contractions of the diaphragm acting under the influence of the automatic respiratory center in the medulla.

Recently, Carlson²² has made the interesting discovery that the heart of the limulus depends for its rhythmic beat on the activity of nerve cells. In this animal the large median nerve stretching along the dorsal surface of the heart contains numerous ganglion cells. When this nerve cord is excised the heart ceases to beat, although still irritable to artificial stimulation. Carlson's work seems to show conclusively that not only the automatic beat of this heart, but the conduction also of the wave of contraction is entirely dependent on the action of the nerve cells in the median nerve cord. We have here, therefore, an instance in which the automatic contractions and the co-ordination of a genuine blood heart are undoubtedly of neurogenic origin. The question arises whether we are justified in applying this result, obtained on an invertebrate heart, to the hearts of the vertebrate animals.

One consideration which presents itself in this connection tends to make an impartial observer doubt whether such a wide induction is permissible. The cardiac muscle in the vertebrate

²¹ Greene: Amer. Jour. of Physiol., 1900, vol. iii, p. 366.

²² Carlson: Amer. Jour. of Physiol., 1904, vol. xii, p. 67; *ibid.*, 1905, vol. xii, p. 471.

heart possesses certain peculiar properties which serve to distinguish it from the usual skeletal muscle. The most characteristic and fundamental of these properties is a long refractory period. That is to say, when cardiac muscle contracts spontaneously, or in consequence of an artificial stimulus, it is entirely unirritable to further stimulation during most of its period of shortening. This reaction is shown by the musculature in all parts of the vertebrate heart, and most physiologists regard it as a property which lies at the basis of the phenomenon of rhythmicity. Now the musculature of the heart of the limulus, of the closely related heart of the lobster, and of the hearts of other invertebrates, does not possess this characteristic property.²³ It is, therefore, to all appearances, a kind of muscle different from the cardiac muscle of the vertebrates, and resembles rather skeletal or voluntary muscle. Like this latter form of muscle it does not possess the property of automatic rhythmicity, but receives its stimulating impulses from the nervous system. Unless it can be shown that the refractory period is not a characteristic and distinguishing property of cardiac muscle as it exists in the higher vertebrates, this discovery that the heart beat of the limulus has a neurogenic origin fails to have a direct bearing on the problem that we are considering, namely, the myogenic or neurogenic nature of the heart beat in vertebrate animals.

An effort to show this very thing has been made within the last few months in a paper published by Rohde.²⁴ The author states that when a frog's heart is dosed with chloral the muscle loses all of its characteristic properties and resembles ordinary skeletal muscle. By paralyzing completely the intrinsic nervous system the chloral gives us a chance to study the heart muscle itself, and according to this paper the muscle under these conditions turns out to be like that of the heart of the limulus or other invertebrates. From this point of view, there-

²³ Carlson: *Amer. Jour. of Physiol.*, 1905, vol. xii, p. 492; also Hunt, Bookman and Tierney: *Centbl. f. Physiol.*, 1897, vol. xi, p. 274.

²⁴ Rohde: *Archiv. f. exper. Path. u. Pharm.*, 1905, vol. liv, p. 104.

fore, what we have called the characteristic properties of heart muscle, the refractory period for example, are really properties of the intrinsic nervous apparatus. It is evident at once to a physiologist that a conclusion of this sort lands us in a difficulty of interpretation to which the author has paid no attention. One may ask such a question as this: If the refractory period is a property of the intrinsic nerves, and if the heart muscle is independently irritable to artificial stimulation, how does it happen that an electrical stimulus applied directly to the muscle of the normally beating heart during the phase of systole fails to provoke a contraction? A seemingly unanswerable question of this kind would not, of course, invalidate actual experimental results, and in view of the importance of the far-reaching conclusions of the author his experiments have been repeated in my laboratory by Mr. Schultz.

The results of this work will be published shortly,* but I may say that they show conclusively that Rohde was in error in saying that chloral effaces all of the characteristic properties of heart muscle. It modifies in an interesting way the response of this muscle to repeated stimulation, especially in the matter of its tone contractions,²⁵ but the striking peculiarity of the heart muscle, namely, the refractory period, is still retained. It is difficult to understand how Rohde could have reached a different conclusion, unless indeed he was misled by an inaccurate method of registration. The objection that I have made to the general application of Carlson's results on the heart of the limulus retains, therefore, its full significance and prevents us from accepting this work as giving a final solution to the problem. Some further facts which tend to support the myogenic view may now be considered.

The Reversal of the Beat.—Under various conditions the beat of the heart may be reversed, that is, the wave of contraction may begin in the ventricles, proceed thence to the auricles, and finally to the sinus venosus. As Gaskell, Engelmann and others

* See Amer. Journ. of Physiol.

²⁵ In this connection see Porter: Amer. Jour. of Physiol., 1905, vol. xv, p. 1.

have pointed out, this reversal, while easily understood on the myogenic theory, is opposed to the usual form of the neurogenic hypothesis. A nervous mechanism, consisting of a principal motor center in the region of the sinus and subordinate motor centers in auricle and ventricle, can not, according to our experience with such mechanisms in other parts of the body, work in both directions; a system of connecting neurons is a mechanism that conducts and co-ordinates only in one direction.

To account for this phenomenon the neurogenists are obliged to assume that the nervous apparatus in the heart forms a peculiar interconnecting network, the like of which is not found in the other automatic nervous mechanisms of the body, not even in those of the intestines. For when any portion of the intestine in a condition of rest is stimulated at a given point the wave of contraction or of contraction and inhibition proceeds onward in normal fashion; the movement is a peristalsis and not an antiperistalsis. In the heart, on the contrary, when at rest, any adequate stimulus applied to the ventricles will set up a reversed rhythm. The necessity forced on the neurogenists to make a new and unproved assumption to meet this case, does not, of course, strengthen their side of the argument. So, too, the well known zigzag experiment by Engelmann fits well into the myogenic theory, but is difficult of explanation in terms of the neurogenic hypothesis without recourse to the unknown properties of a nerve network. In this experiment it was shown that when a ventricle is so cut as to form an irregular piece, with intervening narrow bridges, a stimulus applied at either end arouses a wave of contraction that spreads in orderly sequence over the whole piece.

The Auriculoventricular Bundle.—It was formerly held that the myogenic theory is inapplicable to the mammalian heart because no muscular connection exists between auricles and ventricles. This objection has been completely removed in recent years.

On the anatomical side the work of Kent, W. His, Jr., Retzer, Braeunig, Humblet and Tawara has shown beyond doubt that a small muscular slip passes from the auricle into the ventricular

septum. In man, according to Retzer, this bridge is about 1.5 mm. in thickness, 2.5 mm. in width and 18 mm. long.

On the physiological side the experiments of His, Hering, Humblet, Fredericq and especially of Erlanger, have proved with equal certainty that it is along this narrow bundle that the wave of excitation is conveyed from auricle to ventricle. The last named observer has shown in a series of brilliant experiments that if this bridge be compressed by a specially devised clamp, the sequence of the ventricular on the auricular contractions may be removed either completely or partially. In the former case there is complete heart block, and the ventricle, after a preliminary pause, beats with a slow rhythm entirely independent of that of the auricle. In the latter case the block is partial, and the ventricular beats exhibit a 1 to 2 or 1 to 3 rhythm, as compared with those of the auricle.

Erlanger has succeeded in showing that in man under certain pathological conditions an exactly similar condition prevails, forming the important feature of the Stokes-Adams syndrome.²⁶

Autopsies, indeed, have shown that in some of these cases a demonstrable lesion exists in the region of the bundle. No one can deny the importance of this bundle as the physiological link connecting auricles and ventricles. If it so happened that the tissue composing it was entirely devoid of nerve fibers the myogenic hypothesis would be practically demonstrated. According to Tawara, however, the bundle is provided with a nerve network similar to that found enveloping the muscular tissue of the rest of the heart, and naturally the neurogenists attribute to this network the functions that the myogenists would assign to the muscular bundle itself.

A definite answer to our problem is, therefore, again postponed. The myogenist may, however, urge with justice that the probabilities here are once more in favor of his view. This bundle constitutes the only known muscular connection between auricles and ventricles, while nerve connections between the two

²⁶ Erlanger: The Jour. of Exper. Medicine, 1905, vol. vii, No. 6; *ibid.*, 1906, vol. viii, No. 1.

chambers exist freely in other parts of the auriculoventricular ring²⁷; yet severance of this small muscular bridge is all that is necessary in order to interrupt completely the physiological connection between auricle and ventricle. Investigation of this interesting structure has but just begun. We may hope that future work will develop facts of fundamental significance for the physiology and pathology of the heart. Already one suggestion has arisen out of the work, which indicates the possibility of a new point of view regarding the cause and sequence of the heart beat. Tawara maintains that the cells composing the bundle are not ordinary heart muscle, but that variety of cardiac muscle which has been designated as Purkinje fibers or cells. He believes that the bundle after entering the ventricle spreads out to constitute the Purkinje cells that are known to form a layer beneath the endocardium, and one may conceive that this layer has a still further distribution within the mass of the heart musculature. There is thus presented the possibility of a widespread occurrence of a specially modified type of contractile tissue which may be intimately connected with the phenomenon of automatic rhythmicity as well as conduction. We must, however, await further investigation before attempting to speculate on this modification of the myogenic theory.

The Action of the Accelerator Nerves.—It has been known that the hearts of both warm-blooded and cold-blooded animals may be kept alive for hours after excision from the body, provided they are supplied with an artificial circulation.

Recently the possibilities of thus maintaining an isolated heart, or of reviving its activity after death, have been developed in a remarkable way. Kuliabko was able to restore the beat of the heart in animals that had been dead three or four days, by the simple process of supplying the coronary vessels with a Ringer's solution. He obtained also similar successful results on human hearts as late as twenty hours after death. In an experiment made on the heart of a man who had been dead eleven hours, Hering was able to restore its beat for a

²⁷ See Lomakina: Zeits. f. Biol., 1900, vol. xxxix, p. 377.

period of several hours. Hering has made use of this possibility to study the source of the heart's automatic rhythm.²⁸ He found in the course of experiments on the isolated mammalian heart that the inhibitory and accelerator nerves continued to give their respective effects for a long period of time. In one case in a monkey the vagus retained its inhibitory action for six hours after death, and the accelerator for more than fifty-three hours. On the other hand, comparative experiments made on rabbits and dogs showed that sympathetic nerve ganglia, such as the superior cervical or the ciliary, lose their irritability very quickly after death, even when supplied with an artificial circulation of Ringer's solution. It seems probable from these experiments that the maintenance of an automatic rhythm in the heart so long after excision or after somatic death can not be due to the activity of intrinsic ganglion cells. Since, moreover, the accelerator fibers retained their irritability in these experiments for very long periods after death it would seem probable that they do not end in the ganglia of the heart but are distributed rather directly to the heart muscles. Such a conclusion implies that the rhythm of the heart beat originates in automatic processes within the muscular tissue itself.

THE MYOGENIC THEORY OR THE NEUROGENIC THEORY?

From consideration of this brief review of the current literature and discussions on this subject it appears to me that every impartial observer will be forced to come to the same conclusion as that reached by Hofmann in his excellent critical paper published in 1898, namely, that the myogenic theory is the most probable of any that have been proposed so far. The theory in its most general form assumes that contraction waves or excitation waves arise in the sinus region and are conducted by the muscular tissue over the whole heart, the visible effect at each point being dependent on the condition of the musculature at that moment. At the passage from auricle to ventricle there is a slowing of the conduction due to the small size and

²⁸ Hering: Pflüger's Archiv., 1903, vol. xcix, p. 253.

special properties of the narrow bundle connecting the two chambers. Moreover the condition of the musculature at any point may be influenced in opposite directions by nervous influences, inhibitory and acceleratory, which, however, have nothing to do directly with the origination or conduction of the initial motor impulse.

This theory gives in general an adequate explanation of the phenomena of the normal heart beat, and of those variations that occur under pathological and experimental conditions, but in order to apply it in detail we need to know more of the processes that lead to contraction and relaxation. In fact, the phenomenon of the co-ordination of the beat is not sufficiently accounted for, whether we adopt the myogenic or the neurogenic theory. The difficulty is apparent if we stop to consider those conditions which lead to inco-ordinated contractions, such as are exhibited in the peculiar fibrillated movements of auricle or ventricle. In this condition the mass of the musculature of the ventricle, instead of contracting simultaneously or in a rapid wave running from one end to the other, exhibits feeble local contractions and dilatations which involve only small areas, and give the entire ventricle the appearance of a fluttering, trembling mass.

One of the most remarkable means of thus throwing the co-ordinated contraction of the ventricles into inco-ordinated fibrillary movements is the heart puncture as described by Kronecker and Schmey.²⁹ A needle thrust into the heart at the lower border of the upper third of the septum may produce the transformation almost instantaneously. In my experience the phenomenon is somewhat difficult to obtain. In many cases the heart may be punctured a number of times in the region indicated with no other result than a temporary acceleration of the beat. At other times, however, the first thrust of the needle is followed by the development of fibrillary contractions. Kronecker's first explanation that the needle penetrates a co-ordinating nerve center situated in this part is, as we have seen,

²⁹ Kronecker: *Zeits. f. Biol.* (jubilee volume), 1896, p. 529.

not supported by other facts; and his second suggestion that the phenomenon is due to excitation of a vasomotor center which causes an ischemic condition of the ventricle is likewise difficult to accept, in view of the small evidence that we possess of the existence of vasomotor nerves to the cardiac vessels.

It is, I believe, equally impossible to furnish an entirely adequate explanation of the phenomenon on the myogenic theory, although possibly, a fuller understanding of the properties of the tissue composing the auriculoventricular bundle may throw some light on it. In the terrapin I have seen a similar phenomenon occur in the auricles as a result of stimulation of the vagosympathetic nerve, when the heart was being irrigated with a solution containing an excess of calcium chlorid (0.138 per cent.) or a solution which was lacking in potassium salts. The fibrillation in the former case took place at the end of the stimulation, that is, after the inhibition had passed off.³⁰ It may be, therefore, that the fibrillary contractions are the result of those influences which lead to the development of an augmented rhythmicity throughout the muscular tissue. In the present condition of our knowledge, however, it is perhaps wiser not to speculate on the cause of this singular phenomenon.

³⁰ Dr. Erlanger tells me that in several cases he has observed that stimulation of the vagus nerve in the dog, during experiments in which the chest was widely opened and the pericardium was dissected off, was followed after interruption of the stimulation by a period of incoordinated contractions. McWilliam (*Jour. of Physiol.*, 1888, vol. ix, p. 392) records that in the isolated heart of a cat stimulation of the vagus caused rapid fluttering movements of the auricles instead of the usual inhibition. Knoll (*Pflüger's Archiv.*, 1897, vol. lxxvii, p. 591) refers also to the fact that in the mammalian heart stimulation of the vagus may call forth fibrillary movements in the auricles. Direct stimulation of the heart with induction shocks, if strong enough, will throw the heart into fibrillary movements and a similar result follows or may follow conditions of marked anemia. In strips of the ventricle from the terrapin's heart it is sometimes observed that excessive doses of calcium salts in the bathing liquid may so change the irritability of the muscle that a simple induction shock calls forth fibrillary movements instead of the usual contraction (Schultz).

THE FUNDAMENTAL QUESTION.

Whichever of the two opposing theories we may adopt there remains for discussion the further deeper question of the initial cause of the heart beat. Under normal conditions it will be remembered that the beat arises spontaneously in the sinus region, the rest of the heart contracting in turn only as a result of the stimulus received from the venous end.

The Automaticity of the Heart Beat and the So-called Inner Stimulus.—It has been customary to refer the cause of the spontaneous beat to the production of an inner stimulus, and we have to consider what suggestions have been offered as to its nature and origin. Haller assumed that the venous blood excites the heart, acting presumably as a chemical stimulus. This conclusion seems to have rested mainly on the observation that the heart ceases to beat when deprived of blood. Those who advocated this view made no hypothesis as to the special constituents of the blood which are charged with this important function. Later some of the older observers suggested that the blood acts simply as a mechanical stimulus to the heart, its pressure on the heart walls being the initial cause of the beat.

The experimenters of the early part of the nineteenth century had abundant opportunities to observe that in the cold-blooded animals the heart continues to beat for a long time after removal from the body and even when its cavities are widely opened by incisions. This fact influenced most of the physiologists throughout the nineteenth century to take the stand that the blood has no direct influence on the production of the heart beat. While it furnishes nutriment to the heart as to the other tissues, it is not immediately concerned in the causation of the beat. On the contrary it was assumed that the inner stimulus is autoethonous, that is, arises within the heart itself as a result of its own metabolism. The most concrete statement of this point of view is found in the aphorism used by Langendorff, "*Das Lebensprodukt der Zelle ist ihr Erreger.*"³¹ That is to

³¹ Langendorff: Archiv. f. Anat. u. Physiol. (physiology division), 1884, supplementary volume, p. 1.

say the normal catabolism within the heart muscle or its intrinsic nerve cells gives rise to some substance which acts as a stimulus. There is no evidence, however, other than the automaticity itself, that such a stimulating substance is produced and no suggestion is made as to its nature. That a substance of this sort is not produced during the specific catabolism leading to the contraction is indicated by the fact that in a heart brought to rest by the inhibitory nerve the inner stimulus continues to increase during the period of quiescence, until at last it breaks through the inhibitory control and the heart again beats.

Those who hold to this view, therefore, must assume that this unknown stimulating substance is a product of the resting metabolism of the heart. This, in fact, is the view advocated by Engelmann.³² He holds that there is a continual production of stimulating substance at the sinus end of the heart, which as soon as it reaches a certain quantity excites the muscle and starts a wave of contraction. Since, moreover, each systole is followed by a period of inexcitability which constitutes the diastolic or resting phase, it is necessary in this theory to assume that the process of contraction in some way arrests the production of stimulating substance and indeed destroys or antagonizes that already formed. Hering³³ seems to adopt a similar point of view. Without venturing on any speculations regarding the nature of this stimulating substance or the process leading to its production, he develops the idea that a similar process may occur in the atrioventricular region or in the ventricle itself, the effective stimulus produced in these latter locations being designated as heterotropic in contrast with the normal stimulus which arises at the venous end. In other words he adopts the view that parts of the heart other than the sinus region may develop automaticity under certain conditions.

Many investigators have been unwilling to remain content

³² Engelmann: *Archiv. f. d. ges. Physiol.*, 1897, vol. ixv, p. 109.

³³ Hering: *Centbl. f. Phys.*, 1905, vol. xix, p. 129.

with such general explanations and have sought, therefore, to ascertain what substance or substances constitute the actual stimulus for the heart's contractions, or bear such a close relation to this phenomenon as to form a necessary step in its development. Most of the investigators in question have directed their attention to a careful examination of the specific influence of the individual constituents of the blood. They have followed the general line laid down by Haller in seeking the immediate cause of the heart's activity in the action of the blood itself. The fact that an apparently bloodless heart continues to beat is no argument against such a view, for it is obvious that a heart whose cavities have been depleted of blood by washing is still saturated throughout its substance with tissue lymph, which must be considered in this connection as an actual part of the blood.

One somewhat peculiar view of the relations of the blood to the heart beat has been proposed by Kronecker. As far back as 1874 ³⁴ this author expressed the opinion that the heart muscle can continue to contract only when it receives a constant supply of fresh food material. Subsequently in a series of papers published by himself and his pupils ³⁵ he attempted to show that the serum albumin contained in the blood (and lymph) forms the immediate source of the energy of the heart's contractions, and that these contractions can continue only as long as a supply of this material is present in the liquid bathing the tissue. The insufficiency of the experimental data on which this hypothesis was based has been demonstrated by others ³⁶ and the progress of investigation in recent years has been such as practically to remove it from the field of discussion. At the time that Kronecker's chief investigations were

³⁴ Ludwig's Festgabe, 1874.

³⁵ Kronecker and McGuire: *Archiv. f. Anat. u. Physiol.* (physiology division), 1878, p. 321; also von Ott: *Ibid.*, 1883, p. 1; Kronecker and Popoff: *Ibid.*, 1887, p. 345; Brinck and Kronecker: *Ibid.*, 1887, p. 347; White: *Jour. of Physiol.*, 1896, vol. xix, p. 344.

³⁶ See Howell: *Amer. Jour. of Physiol.*, 1898, vol. ii, p. 47; also Greene: *Ibid.*, p. 82.

made the importance of the effect of the inorganic constituents of the blood was not adequately understood.

Recent Investigations.—The most modern and seemingly the most hopeful line of investigation on the cause of the heart beat was started by Ludwig in 1875. Under his guidance Merunowicz began the study of the relations of the inorganic constituents of the blood to the heart beat. Before that time and for many years subsequently, perhaps even at the present day, physiologists were accustomed to consider only or mainly the organic constituents of the heart or of the blood in discussing the cause and conditions of the rhythmic beat. In the theories of Langendorff and of Engelmann referred to above it is implied, although not specifically stated, that the unknown substance constituting the “inner stimulus” is organic in nature. Merunowicz was able to show that aqueous solutions of the ash of blood, free from all organic matter, have a remarkable efficacy in causing and maintaining the contractions of the frog’s heart.³⁷ He attempted to analyze the specific influence of the several constituents of the blood ash, but in this effort he and the workers in the same laboratory who continued his investigations, were unfortunate in overlooking the importance of the minute amounts of calcium salts present.³⁸ Nevertheless it was made clear by their work, particularly by that of Merunowicz, that the inorganic constituents of the blood have an important relationship to the development of the heart beat.

In 1883, Ringer, by a fortunate accident, was led to study the effect of calcium salts on the heart’s contractions,³⁹ and on the basis of his experimental results he devised an artificial serum, known now as Ringer’s solution, which is capable of maintaining the beat of the heart in a wonderful way. This solution contains certain amounts of sodium, potassium and calcium salts, and for some hearts also a trace of alkali in the form of

³⁷ Arbeiten aus d. phys. Anstalt in Leipzig, 1875, p. 132.

³⁸ Gaule: Archiv. f. Anat. u. Physiol. (physiology division), 1878, p. 291; also Stiénon: *Ibid.*, 1878, p. 263.

³⁹ Ringer: Jour. of Physiol., 1883, vol. iv, p. 29 and p. 370.

sodium carbonate. Its extraordinary efficiency in developing and maintaining the beat of the isolated heart both in the cold-blooded and the warm-blooded animals is known to all physiologists. The heart of the dog, rabbit, cat and of man himself has been kept beating on this inorganic diet for many hours, or even for days. Locke⁴⁰ has succeeded recently by making use of a modified Ringer's solution, containing dextrose, in keeping a rabbit's heart beating for four days after removal from the body. The action of this artificial serum and the specific effects of its different constituents have since been the object of a large number of investigations by Ringer himself and by many other workers in various parts of the world. It is entirely evident that a simple aqueous solution containing only sodium, calcium and potassium salts, together with oxygen, cannot furnish nutriment to the heart in the sense of supplying it with a source of energy for its contractions. The rôle of these salts must be found in their relations to the origination of the heart beat, that is to say, their connections with the chemical changes in the organic constituents which give rise to the energy of the contraction.

Experiments on the hearts of the cold-blooded animals indicate that so far as the circulating liquid is concerned the potassium salts do not form an absolutely necessary constituent. The heart as a whole, or different parts of it, may continue to give rhythmic beats when immersed in or supplied with a solution containing only sodium and calcium salts. There is no doubt, however, that for the heart as a whole, and, therefore, especially for the venous end of the heart, the presence of potassium salts makes a better balanced mixture, and one which maintains a normal beat for a longer period of time. We must bear in mind that the heart muscle contains a considerable store of potassium, and the fact that it continues to contract rhythmically when supplied with a mixture containing only sodium and calcium salts, is no proof that the potassium does not continue to play its important and necessary rôle, whatever

⁴⁰ Locke: *Centbl. f. Physiol.*, 1905, vol. xix, p. 737.

that may be. All the evidence from experimental work indicates that the potassium salts are concerned chiefly with the production of the condition of relaxation, the phenomena of diastole and inhibition.⁴¹ In the matter of the contraction of the heart, therefore, the rôle assumed by the sodium and the calcium respectively, has excited the chief interest. One set of observers (Loeb, Lingle⁴²) have believed that the sodium salts stand in the most immediate relation to the process originating the contractions. "Among the ions found in blood, those of sodium are the producers of rhythmic activity. They constitute the primary stimulus." While others (Ringer, Howell, Langendorff, *et al.*) believe that it is the calcium salts which are most directly concerned in the actual contraction, although the manner and extent of their action are differently interpreted by the several authors. A large literature has sprung up in recent years on the specific effects of these salts under different conditions.

The time at my disposal does not permit me to enter into the details of the discussion, and, indeed, the facts at present are not so numerous nor so definite that one is able to draw any very satisfactory conclusions in regard to their real manner of action. Instead of attempting to present or discuss these details permit me to call your attention to certain general conclusions that may be derived from the accepted fact that these inorganic elements play an essential part of some sort in the processes underlying the heart beat.

General Conclusions.—In the first place what are or may be their relations to the so-called inner stimulus? Langendorff⁴³ and others while admitting the necessity of these substances, characterize them simply as conditions which must be supplied in order that the inner stimulus may act; conditions of the same general character, for example, as a suitable temperature

⁴¹ See Howell: Amer. Jour. of Physiol., 1906, vol. xv, p. 280.

⁴² See Lingle: Amer. Jour. of Physiol., 1900, vol. iv, p. 265.

⁴³ See *Ergebnisse d. Physiol.*, vol. i, part ii, 1902; also vol. iv, part ii, 1905.

or the presence of oxygen. From this standpoint, therefore, the inorganic salts have nothing to do directly with the initiation of the beat, although this phenomenon can not take place in their absence. On the other hand Lingle has stated that the sodium ions form the direct stimulus to the heart's activity, while I have been quoted by several authors as advocating the view that the calcium ions constitute the inner stimulus. I must object to this interpretation of my views on the action of the calcium salts. In my last paper on the subject I have stated simply "that the energy of the heart beat is derived from material stored in its own substance. For the utilization of this supply of energy, however, certain conditions are necessary, and the principal one of these conditions seems to be the presence in the liquids of the heart of a supply of calcium in some form." And again, "Under normal conditions the stimulus that leads to a heart contraction is dependent on the presence of calcium compounds in the liquids of the heart."

These views do not differ materially from those finally accepted by Langendorff, except in the fact that he supposes the creation in the heart of some as yet unknown substance which acts as the "inner stimulus." We have no proof that any such substance is formed and, indeed, to my mind there is no necessity for assuming the existence of a specific inner stimulus. In discussing such a point one must define first of all what is meant by a stimulus. Ordinarily by this term we mean some form of energy which, acting on the irritable living substance, causes it to exhibit its specific form of activity. If the chemical reaction underlying functional activity is directly induced by a special compound or even by a special ferment, I presume that we should be justified in characterizing this compound or this enzyme as a stimulus. But if, as may well be the case, the living substance is of sufficient instability to break down spontaneously, under the conditions prevailing in the tissue, then the chain of events leading to the display of functional activity may be inaugurated without the occurrence of a specific inner stimulus. It is in this manner that I conceive that the spontaneous beat of the heart arises.

The immediate cause of the contraction is a chemical reaction or a series of such reactions. In accordance with the knowledge of our day we may assume that the first step in this series consists in the dissociation, the falling into pieces of a complex, unstable molecule, and that this dissociation is followed by an oxidation of the split products. The undoubted necessity of the oxygen for the normal production of a heart contraction may be referred, therefore, to the part that it plays in the second stage of the process, and not to its action as a primary or initial stimulus. The "inner stimulus," if such a thing exists, must be concerned in the production of the initial step of dissociation. We may inaugurate this first step, as is well known, by the application of some external form of energy, such as a mechanical impulse, an electrical current, a nerve impulse, etc., and it is conceivable, of course, that it may be started, as Langendorff and Engelmann have supposed, by some specific substance formed in the metabolism.

It seems to me, however, equally as probable or more probable that this initial step takes place really automatically or spontaneously, in consequence of the instability of the substance in question. My own work has convinced me that the calcium salts are in some way of prime importance in this matter of the initial dissociation of the energy-yielding substance, but I do not believe that they act as a direct chemical stimulus. Speculations on this subject must at present go beyond the limits of our real knowledge and are, therefore, liable to be partly or completely erroneous.

The following facts, however, must be taken into account by any hypothesis which attempts to picture the processes causing the rhythmic contraction and dilatation of the heart muscle:

1. The heart possesses within itself a store of energy-yielding material, such that it may continue to give many hundreds or thousands of contractions after its supply of nutriment has been cut off.

2. Each contraction, whether caused normally or by an artificial stimulus is maximal, and, therefore, probably uses up all of the energy-yielding material which is at that moment in an

irritable condition, that is to say, in such a condition that it may be acted on by a stimulus.

3. The amount of this material in irritable form is nil during the phase of systole, but increases in amount throughout the period of diastole. We know, for example, that if stimulated just at the beginning of diastole the heart muscle gives a small contraction and that the contractions which may be obtained later by artificial stimulation increase in extent the farther the diastole has progressed.

4. If the above statements are correct it follows that the store of energy-yielding material in the heart exists in some non-irritable form and that during the phase of diastole a portion is converted into an irritable form capable of being acted on by a stimulus.

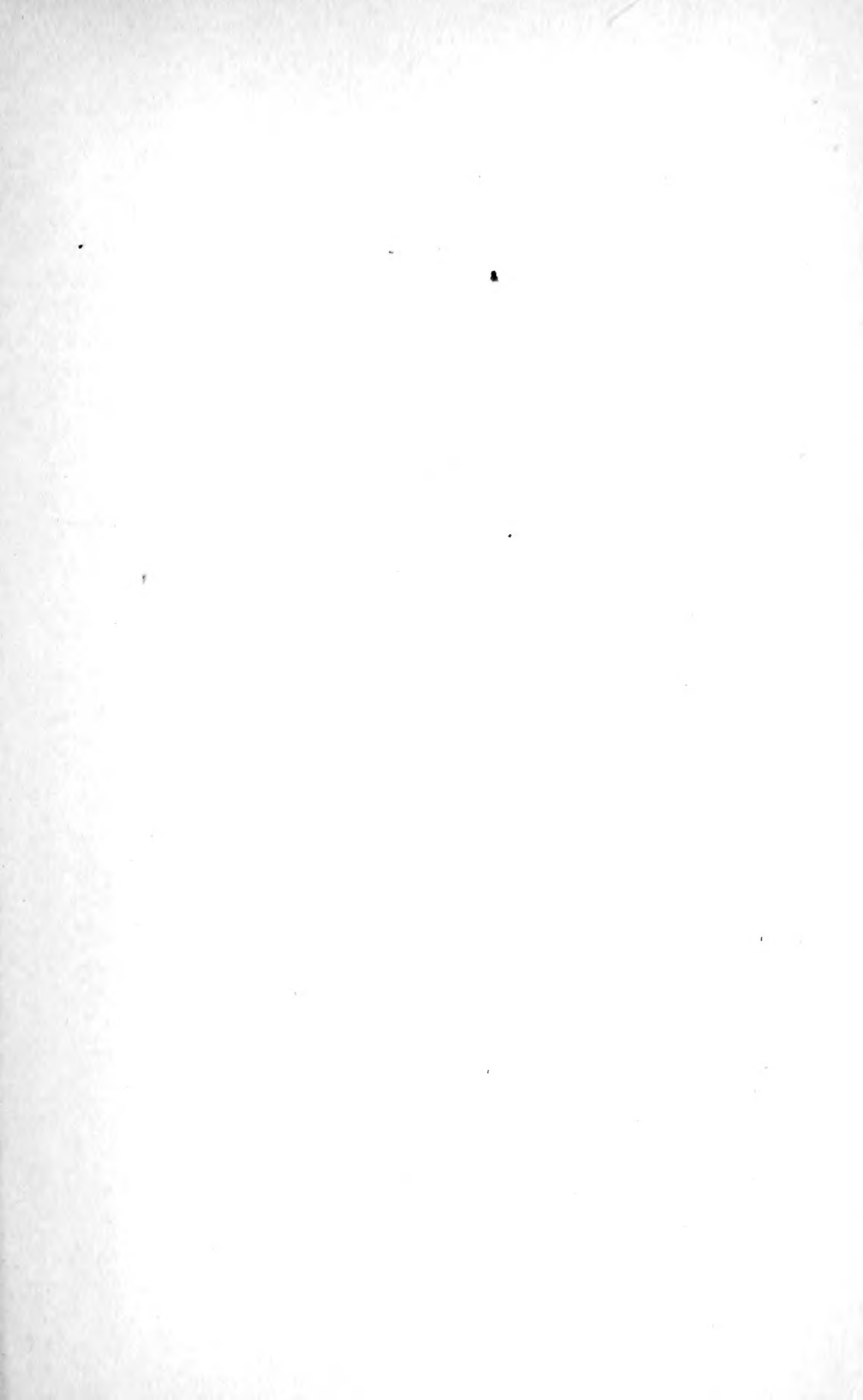
5. The presence of certain inorganic salts is necessary for this transformation from the non-irritable to the irritable condition.

In order to picture the relations of the inorganic salts to this process the hypothesis which I have adopted as a heuristic principle to guide my own investigations, may be stated as follows: The well-nourished heart contains a large supply of energy-yielding material, which is in stable form, so that it neither dissociates spontaneously, nor can be made to do so by the action of external stimuli. It is possible that this stable, non-dissociable form consists of a compound between it and the potassium or the potassium salts and that herein lies the functional importance of the large amount of potassium contained in the tissue. This compound reacts with the calcium or with the calcium and sodium salts, and a portion of the potassium is replaced and a compound is formed which is unstable. At the end of the diastolic period this compound reaches a condition of instability such that it dissociates spontaneously, giving rise to the chain of events that culminates in the normal systole. This dissociation may be made to take place prematurely by an external stimulus, such as a mechanical or electrical shock applied to the heart at any time after diastole has begun.

From this point of view the rôle of the calcium, or of the calcium and sodium salts, consists in replacing the potassium and converting a part of the store of stable material into an unstable, easily dissociable compound. We are not obliged, therefore, to assume the existence of any specific inner stimulus. An hypothesis of this character accounts readily for some of the most characteristic features of the heart beat.

Each contraction must be maximal since it involves the dissociation of all the material existing in unstable form. The contractions must be rhythmic since, after each contraction, a certain interval, which will be constant when the conditions are uniform, is needed for the production of more of the unstable material. At each systole the heart will exhibit a refractory phase, since the ready-formed, unstable material has been used up and the rest of the energy-yielding substance exists in a stable, non-irritable form. In terms of the hypothesis the refractory phase should pass off gradually as new, unstable material accumulates, and this we know to be the case, since a weaker stimulus is required to force the heart to contract the later it is applied in the diastolic phase.

Whether or not this or any other of the hypotheses described turns out to be correct, we may congratulate ourselves at least that the labors of the experimental physiologists during the last quarter of a century have added to our store of knowledge this new and important fact, namely, that the inorganic salts of the blood and lymph play an essential rôle in the production of the heart beat.



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